Chapter 3

Imparting Anti-tumor Specificity to the Mouse T Cell Repertoire

1. Introduction

The naturally occurring T cell repertoire in an individual is composed of up to $1x10^{12}$ T cells expressing some $2.5x10^{7}$ T cell receptors (TCRs), with each T cell bearing TCRs of a single specificity (Arstila et al., 1999). The enormous size of the T cell repertoire allows an animal to respond to a wide diversity of antigens. During an immune response, the antigen-specific T cell clones can rapidly expand. "Clonal expansion" is the hallmark of adaptive immunity, and provides an efficient way for the adaptive immune system to protect organisms against infectious diseases.

While the immune system handles most pathogens well, it does a poor job of suppressing the growth of tumors. This phenomenon is not totally understood, but much evidence suggests that the limited number of T cells capable of responding to tumor cells, insufficient avidity of these T cells for tumor antigens, and tolerogenic attenuations by the tumor contribute to this immunological failure (Chambers et al., 2001; Marincola et al., 2003; Matzinger, 2002; Ochsenbein, 2002; Smyth et al., 2001). Therefore, a major goal of cancer immunotherapy has been to generate a large number of highly tumor-specific T cells that can last *in vivo* for a long time and resist tolerization (Rosenberg, 2001). Existing methods focus on reshaping the normal T cell repertoire, and fall into two categories: active expansion of the endogenous anti-tumor T cell clones by immunization (Berzofsky et al., 2001; Finn, 2003) and passive (adoptive) transfer of T cells with anti-tumor reactivity (Dudley and Rosenberg, 2003; Ho et al., 2003). Genetically enhancing the adoptively-transferred T cells to improve their anti-tumor efficacy

is also under active investigation (Pardoll, 2002a; Pardoll, 2002b; Sadelain et al., 2003).

We propose that directly imparting anti-tumor specificity to the normal T cell repertoire could provide a new opportunity for increasing the anti-cancer effectiveness of the immune system. The conventional TCR transgenic method can achieve this via pronuclear injection of pre-rearranged TCR genes (Vonboehmer, 1990). The majority of the T cell repertoire generated in the resultant mice will carry the specificity of the transgenic TCR due to the allelic exclusion in T cell development (Vonboehmer, 1990). But this method offers no opportunity for therapeutic application in humans. By contrast, hematopoietic stem cells (HSCs) are attractive targets for genetic modification due to their roles as T cell progenitors, their enormous biological capacity and their availability for modification in humans (Morrison et al., 1995). In this report we describe a method to impart the desired anti-tumor specificity to the mouse T cell repertoire by delivering tumor-specific TCR genes into HSCs, followed by adaptive transfer to generate a continuous stream of anti-tumor T cells in host mice.

2. Materials and Methods

Mice

C57BL/6J(B6) female mice were purchased from Charles River Breeding Laboratories, and RAG1 deficient female mice in the B6 background were purchased from The Jackson Laboratory. All mice were housed in the California Institute of Technology animal facility in accordance with institute regulations.

MOT1 Retrovirus

The MOT1 construct was generated from the MIG retrovirus (Van Parijs et al., 1999) by replacing GFP with the OT1 TCR beta chain cDNA and cloning the OT1 TCR alpha chain cDNA in front of the IRES. Retroviruses were made in HEK293.T cells using a standard protocol (Pear et al., 1993). Viruses were harvested 36-48 hours after transfection.

Primary T Cell Infection and Stimulation

Spleen cells were harvested from B6 female mice and activated *in vitro* with 0.5μg/ml anti-CD3 and 0.5μg/ml anti-CD28 Abs (both from Pharmingen). On day 2 of culture, cells were spin-infected with MOT1 retroviruses in the presence of 10μg/ml polybrene for 90min at 2,500rpm at 30ºC. On day 3, cells were collected for analysis.

Hematopoietic Stem Cells (HSCs) Isolation, Infection and Transfer

B6 female mice or $RAG1^{-/-}$ female mice were treated with 250μg/g of body weight of 5-fluorouracil (Sigma). Five days later, bone marrow (BM) cells enriched with HSCs were harvested and cultured for 4 days in RPMI containing 10% FBS with 20ng/ml rmIL-3, 50ng/ml rmIL-6 and 50ng/ml rmSCF (all from Biosource International). On day 2 and 3, the cells were spin infected with MOT1 retroviruses supplemented with 8μg/ml polybrene for 90min at 2,500rpm, 30ºC. On day 4 of culture, BM cells were collected and transferred by tail vein injection

into B6 female hosts or RAG1$^{-/-}$ female hosts that had received 1200 rads or 360 rads whole-body radiation. Each host received 2-3x10^6 infected BM cells. BM recipient mice were maintained on the mixed antibiotic sulfmethoxazole and trimethoprim oral suspension (Hi-Tech Pharmacal, Amityville, NY) in a sterile environment for 6-8 weeks until analysis or usage for further experiments.

In vitro *T cell Stimulation and Functional Assays*

Spleen cells from BM recipient mice were harvested and cultured at 2x10^5 cells/well in T cell culture medium containing OVAp ($OVA_{257-264}$ SIINFEKL peptide, synthesized at Caltech Biopolymer Synthesis Center) at 0-1μg/ml. Three days later, culture supernatants were collected and assayed for IFN-γ production by ELISA, and proliferation was assessed by [^{3}H]thymidine incorporation as described before (Yang et al., 2002).

Antibodies and FACS Analysis

Fluorochrome-conjugated antibodies specific for mouse CD4, CD8, CD25, CD69, CD62L, CD44, TCRVα2, TCRVβ5.1,5.2 were purchased from BD Pharmingen (San Diego, CA). Surface staining was performed by blocking with anti-CD16/CD32 (mouse Fc receptor, BD Pharmingen, San Diego, CA) followed by staining with fluorochrome-conjugated antibodies. Intracellular staining of TCR was done using the Cytofix/Cytoperm^{TM} Kit from BD Pharmingen (San Diego, CA). Analyses were performed on a FACScan flow cytometer.

T cell Memory Study

Spleen and lymph node cells from BM recipient mice (B6/MOT1) were harvested and stimulated with 0.1μg/ml OVAp for 3 days *in vitro*. The cells were then collected and transferred into $RAG1^{-/-}$ hosts by tail vein injection. Each host received 20-30x10^6 cells (>10% were activated OT1 T cells). Sixteen weeks later, spleen cells were harvested from the hosts and analyzed for the presence of long-lived OT1 T cells. Memory phenotype of the OT1 T cells was studied by FACS. Memory function was studied by antigen dosage response, antigen time-course response and cytokine proliferation response of the OT1 T cells. In antigen dosage response, cells were stimulated with 0-1μg/ml OVAp for 3 days, and the culture supernatants were collected and assayed for IFN-γ production by ELISA; proliferation was assessed by [^{3}H]thymidine incorporation as described before (Yang et al., 2002). In antigen time-course response, cells were stimulated with 0.1μg/ml OVAp, and the culture supernatants were collected and assayed for IFN-γ production by ELISA on day 1.5, day 2.5 and day 3.5. In cytokine proliferation response, cells were cultured with 10ng/ml rmIL-2 or 10ng/ml rmIL-15 (all from BioSource International, Camarillo, CA) for 4 days in the absence of antigen, and proliferation was assessed by [^{3}H]thymidine incorporation.

Tumor Challenge of Mice

The tumor cell lines used were EL.4 (C57BL/6, H-2^b, thymoma) and E.G7 (EL.4 cells transfected with the chicken OVA cDNA)(Moore et al., 1988). 5x10^6

EL.4 or E.G7 cells were injected subcutaneously into the left flank of the mice. Tumor size was measured every other day using fine calipers (Manostat Corporation, Switzerland), and is shown as the product of the two largest perpendicular diameters a x b (mm^2). Mice were euthanized when the tumors reached 400mm^2.

D*endritic Cell Generation, Antigen Pulsing and Mouse Immunization*

Dendritic cells (DC) were generated from bone marrow cultures as described by Lutz MB et al. (Lutz et al., 1999), with some minor modifications. Briefly, bone marrow cells were harvested from B6 female mice and cultured in 10cm diameter petri dishes at $2x10^6$ cells/dish in 10ml R10 medium (RPMI-1640 supplemented with 100U/ml Penicillin, 100µg/ml Streptomycine, 2mM L-glutamin, 50µM 2-mercaptoethanol and 10% FBS) containing 1:30 J558L culture supernatant. J558L is a cell line transfected with the murine GM-CSF gene (Zal et al., 1994) and its culture supernatant was used at the source of GM-CSF. On day3 another 10ml R10 medium containing 1:30 J558L culture supernatant was added into each dish. On day 6 and day8, half of the culture supernatant was collected and centrifuged, and the cell pellet was resuspended in 10ml fresh R10 medium containing 1:30 J558L culture supernatant and added back into the original culture dishes. On day 9, non-adherent cells were collected and plated into new 10cm diameter petri dishes at $4\text{-}6x10^6$ cells/dish in 10ml R10 medium containing 1:60 J558L culture supernatant, in the presence of 1µg/ml LPS (Sigma) to mature DCs. On day10, non-adherent cells (usually >80% are mature

DCs) were collected and washed once with IMDM/50mM 2-mercaptoethanol and resuspended in 0.8ml of the same medium containing 100μg OVAp. The cells were then incubated at 37ºC for 3 hours with gentle shaking every 30min. Three hours later, the OVAp loaded DCs were washed twice with PBS and used to immunize mice by tail vein injection. Each mouse received about $0.5x10^6$ OVAp loaded DCs.

3. Results and Discussion

We chose the E.G7 mouse tumor model to test the feasibility and potential of this method for cancer immunotherapy. E.G7 is a mouse thymoma cell line, which was generated by engineering the parent cell line EL.4 to express the chicken OVA gene (Moore et al., 1988). Previous studies showed that the OVAp epitope (SIINFEKEL) displayed on E.G7 surface can be recognized by the OT1 TCR (Hogquist et al., 1994), a well-characterized mouse CD8 TCR, at a density (100 H-$2k^b$/OVAp per cell) similar to tumor antigens on authentic tumor cells (Rotzschke et al., 1991). Thus, the E.G7 tumor cell-OT1 T cell system has been widely used to study cytotoxic T cell (CTL) mediated anti-tumor immune responses (Helmich and Dutton, 2001; Shrikant et al., 1999).

Our first step was to examine the possibility of generating OT1 CTLs in wild-type B6 mice. Previously, we reported success in generating antigen-specific T cells in RAG1 deficient ($RAG1^{-/-}$) mice by co-infection of RAG1 deficient HSCs with retroviruses expressing TCRα or TCRβ cDNA (Yang et al., 2002). In addition to using HSCs as the target for gene transfer, this approach has

additional features that favor clinical application: small genetic elements (TCRα or TCRβ cDNA) and a convenient viral promoter to drive the expression of transgenes. However, when we used this approach to infect wild-type HSCs with retroviruses expressing OT1 TCRα or β cDNA and transferred the HSCs to wild-type recipient mice, no OT1 CTLs were detected. Analysis showed that T cells generated from the transduced HSCs expressed only one of the OT1 TCR chains (Yang and Baltimore, 2004). This result implies that in the presence of endogenous TCR competition, co-infection is not sufficient to achieve co-expression of TCRα and β chains in HSCs to generate transgenic T cells. In the case of RAG1$^{-/-}$ HSCs, the small population of co-expressing cells could be expanded by selection pressure during T cell development.

This prompted us to test co-expression in a single retroviral vector of α and β chains of OT1 TCR linked by an internal ribosome entry site (IRES). The cDNAs encoding OT1 TCR α and β chains were inserted into a retroviral vector MSCV (mouse stem cell virus) under the control of the viral LTR promoter. The resulting construct was designated as MOT1 (Figure 3-1A). When MOT1 retroviruses were used to infect mature mouse T cells, a high expression of OT1 TCRs on the cell surface was observed, and the infected cells were able to respond to OVAp stimulation to produce the effector cytokine IFN-γ (Figure 3-2).

To test whether anti-OVAp specificity could be added to the T cell repertoire via genetic engineering of HSCs, we harvested HSCs from wild-type mouse bone marrow (BM) and infected them with MOT1 retroviruses. RAG1$^{-/-}$ HSCs were included as a control. The infected RAG1$^{-/-}$ and wild-type B6 HSCs

were transferred into RAG1$^{-/-}$ (RAG/MOT1) and wild-type (B6/MOT1) host mice respectively, and analysis was performed after 7 weeks. A significant percentage (4~5%) of bone marrow cells in the recipient mice were transduced cells, as measured by intracellular expression of OT1 TCR α and β chains (Figure 3-1B). Analysis of surface HSC markers c-kit and Sca-1 showed that some of the transduced cells were HSCs (Yang and Baltimore, 2004). We analyzed the host mice after 8 months post-transfer, and found that the percentage of transduced HSCs remained constant (Yang and Baltimore, 2004). Furthermore, the transduced HSCs persisted through the secondary transfer of the recipient bone marrow cells (Yang and Baltimore, 2004). These findings indicated that HSCs infected with MOT1 viruses were able to maintain the stem cell features of longevity and self-renewal; MOT1 mediated expression of OT1 genes in HSCs was not silenced. Therefore, this genetic method for producing modified T cells is sufficiently robust to ensure the maintenance of an introduced T cell population for the lifetime of the host.

To confirm the existence of a stable source of genetically programmed T cell progenitors, we analyzed thymocytes from the host mice. Owing to a lack of endogenous TCRs, all thymocytes in RAG/MOT1 host mice after 7 weeks (Figure 3-1C) and 8 months (Yang and Baltimore, 2004) post-transfer appeared to express OT1 TCR, and 30% showed high OT1 TCR expression. Normal RAG1$^{-/-}$ thymocytes were unable to progress into CD4 and CD8 double positive stage. As shown in Figure 3-1C, RAG1$^{-/-}$ HSCs expressing OT1 TCR gained the ability to develop into double positive and further into CD8 single positive T cells.

No leakage into the CD4 single positive T cell compartment was observed, indicating a high specificity for this system in the guidance of T cell development to the expected fate. This finding was confirmed by the observation that in the periphery of RAG/MOT1 host mice, no CD4 T cells were found, and all the CD8 T cells apparently expressed OT1 TCR (Figure 3-3C).

When we analyzed the thymus from B6/MOT1 host mice, we observed a significant percentage (4%) of thymocytes expressing OT1 TCR (Figure 3-1C). An augmentation of the CD8 single positive compartment was observed in comparison to wild-type animals (Figure 3-3C). Further analysis of B6/MOT1 peripheral T cells showed that in a representative experiment, 22% of the mature CD8 T cells expressed OT1 TCR, and that no CD4 T cells expressed OT1 TCR. (In numerous experiments, the percentage of OT1 T cells ranged from 15% to 30%, with the average at about 20%). Therefore, our results show that through virus-mediated expression of TCR cDNAs in wild-type HSCs, a significant population of T cells with the desired specificity can be generated. In natural conditions, this percentage can only be achieved by clonal expansion of a few high-affinity CD8 clones after strong immune stimulations (Butz and Bevan, 1998).

This methodology could provide a new therapeutic opportunity by continuously generating a large percentage of T cells against a tumor. We therefore focused our attention on whether the T cells generated by this method are functional. One concern was that the control of TCR expression in these T cells is driven by a constitutively active promoter (the retroviral LTR), instead of

the endogenous TCR promoter. The alteration of the level and timing of TCR expression might affect the behavior of the generated T cells (Vonboehmer, 1990). We performed the following series of experiments to address this question. First, we examined the surface activation markers of the OT1 T cells at different activation stages. Before activation, the naïve OT1 T cells displayed a common naïve phenotype of CD8 T cells with $CD25^{-}CD69^{-}CD62L^{high}CD44^{low}$ (Figure 3-3A, upper panel). Upon stimulation with OVAp, OT1 T cells were activated. FACS analysis showed that the activated OT1 T cells exhibited the typical phenotype of active CTLs: up-regulated CD25, CD69 and CD44 and down-regulated CD62L (Figure 3-3B, middle panel).

We then tested the ability of the OT1 T cells to generate and maintain CD8 T cell memory by adoptively transferring the *in vitro* activated cells to $RAG1^{-/-}$ hosts. Sixteen weeks after transfer, spleen cells were harvested from the adoptive hosts and analyzed for the presence of long-lived memory OT1 T cells. As shown in Figure 3-3A (lower panel), 5% of CD8 cells were OT1 T cells, and displayed the surface phenotype of memory CD8 T cells ($CD25^{-}CD69^{-}CD62L^{high}CD44^{high}$).

In addition to our surface staining results, we further tested the ability of these OT1 T cells to proliferate and produce cytokines upon antigen stimulation. When stimulated with OVAp, the OT1 T cells proliferated and produced the effector cytokine IFN-γ in a dosage-dependent manner, with a magnitude comparable to the response of transgenic OT1 T cells obtained from the conventional TCR transgenic mice (Figure 3-3B). We then tested the ability of

memory OT1 T cells to mount a secondary response. These cells showed a stronger response to antigen stimulation, as indicated by their ability to produce higher levels of IFN-γ compared with naïve OT1 T cells (Figure 3-3C, left). Time course experiments also showed that these memory OT1 T cells responded to OVAp stimulation faster than naïve OT1 cells (Figure 3-3C. middle).

Another behavior of memory T cells we tested is cytokine-induced proliferation, which is a unique feature of memory T cells because the proliferation of naïve T cells is strictly controlled by antigen recognition (Schluns and Lefrancois, 2003; Tanchot et al., 1997). When we measured the responsiveness of OT1 T cells to cytokines, memory OT1 cells proliferated and naïve OT1 T cells did not (Figure 3-3C, right panel).

Taken together, these results show that the OT1 T cells generated using our method are fully functional and normal in all aspects we evaluated. In particular, the ability of these T cells to generate and maintain long-term memory makes our method markedly attractive for immunotherapy.

The key step was to test the potential of our method for cancer immunotherapy in the E.G7 mouse tumor model. As a pilot experiment, we found that OT1 CD8 T cells generated by lentiviral transgenesis could recognize and respond to E.G7 tumor cells *in vitro*, as indicated by IFN-γ production (Figure 3-3B, right panel). When injected subcutaneously into wild-type B6 mice, E.G7 cells grew into large solid tumors. The protocol we used to ascertain if tumor growth could be suppressed by genetically modifying HSCs is outlined in Figure 3-4A. HSCs harvested from B6 donor mice were infected with MOT1

retroviruses and subsequently transferred into syngeneic B6 hosts. The host mice (B6/MOT1) were allowed to reconstitute their T-cell compartment over 8-10 weeks. E.G7 tumor cells were then injected subcutaneously. To evaluate the effects of immunization, 4 groups of mice out of 8 were immunized using dendritic cells loaded with OVAp 4 days after tumor injection. Tumor growth was monitored daily, and mice were euthanized if tumors reached a size of 400 mm^2. Four mice were used in each treatment group, and the experiments were performed three times.

The results of one representative experiment are shown in Figure 3-4B. In B6 control mice that did not receive transfer of MOT1-transduced HSCs, solid tumors grew up 7 days after E.G7 cells injection. In sharp contrast, tumor growth was significantly suppressed in mice receiving transfer of MOT1-transduced HSCs. Without immunization, complete suppression of E.G7 tumor growth was observed in 50% of the mice, with no recurrence of tumors observed as long as the experiments ran (up to 200 days). For the other 50%, tumor growth was suppressed up to 18 days but eventually progressed. We analyzed the OT1 cells recovered from these tumor-bearing mice, and found that the ability of OT1 T cells to mount an immune response to tumor cells was greatly attenuated by an apparently tolerogenic mechanism (Overwijk et al., 2003; Yang and Baltimore, 2004).

We speculated that a booster immunization using dendritic cells loaded with OVAp might enhance OT1 T cells function and thus improve their anti-tumor ability (Cerundolo et al., 2004). As hoped, this booster immunization strategy

thoroughly suppressed tumor growth in all the mice receiving BM transfers of MOT1-transduced HSCs. Importantly, this strategy only worked in mice that had a T cell repertoire with genetically enhanced anti-tumor specificity. In the B6 control mice that did not receive MOT1-transduced HSCs, tumor growth was not detectibly suppressed by immunization. Moreover, the control tumor EL.4 grew up in both B6 control mice and B6/MOT1 mice with an identical growth rate, again independently of immunization. This result confirms that suppression of E.G7 tumor growth is mediated by the generated OT1 T cells that carry the anti-OVAp specificity.

4. Conclusion

In summary, we have successfully developed a method to impart a desired anti-tumor specificity to the T cell repertoire via genetic engineering of HSCs. Using this method, a large population of anti-tumor CTLs can be generated *in vivo*, accounting for more than 20% of the peripheral CD8 T cells. These T cells are fully normal and functional in all aspects we tested, most notably in the generation of long-term memory. When we tested the efficacy of our method in cancer immunotherapy in E.G7 mouse tumor model, we demonstrated efficient suppression of tumor growth.

In the last decade, tumor specific antigens and the TCRs recognizing them have been identified for many human cancers (Boon et al., 1994; Renkvist et al., 2001; Rosenberg, 1999), providing new opportunities for cancer immunotherapy. Building upon this progress, we have combined gene therapy,

stem cell therapy and immunotherapy to create a method that holds promise in treating cancer. We could further extend this method to impart to the T cell repertoire multiple anti-tumor specificities against different epitopes of tumor antigens, thus countering the tendency of tumors towards “epitope escape” by mutation of tumor antigens (Khong and Restifo, 2002). Our approach could further be adapted to treat chronic infectious diseases, such as cytomegalovirus (CMV) and human immunodeficiency virus (HIV) (Riddell and Greenberg, 2000). It could also be used for rapid and efficient analysis of the development and life history of antigen-specific T cells, providing a new tool for basic T cell biology research.

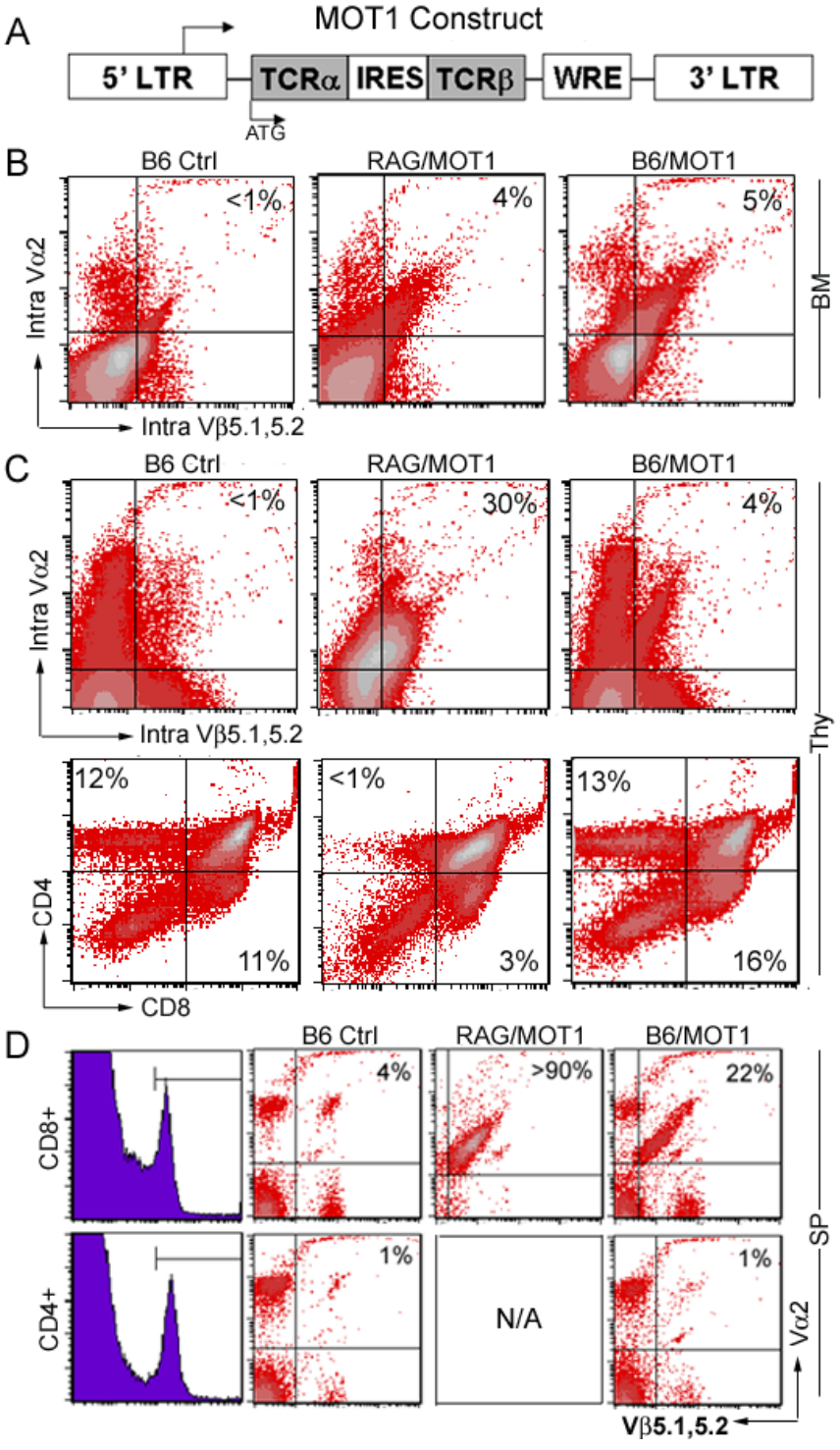
A
MOT1 Construct
5' LTR
TCRα
IRES
TCRβ
WRE
3' LTR
ATG
B
B6 Ctrl
RAG/MOT1
B6/MOT1
<1%
4%
5%
Intra Vα2
Intra Vβ5.1,5.2
BM
C
B6 Ctrl
RAG/MOT1
B6/MOT1
<1%
30%
4%
Intra Vα2
Intra Vβ5.1,5.2
12%
<1%
13%
11%
3%
16%
CD4
CD8
Thy
D
B6 Ctrl
RAG/MOT1
B6/MOT1
CD8+
CD4+
4%
>90%
22%
1%
N/A
1%
Vα2
Vβ5.1,5.2
SP

Figure 3-1

Figure 3-1: Imparting a desired antigen specificity to the mouse T cell repertoire by retrovirus-mediated expression of TCR cDNAs in hematopoietic stem cells (HSCs). HSCs from $RAG1^{-/-}$ or B6 mice were infected with MOT1 viruses and transferred into either $RAG1^{-/-}$ host mice (RAG/MOT1) or B6 host mice (B6/MOT1), respectively. Seven weeks later, host mice were analyzed for OT1 T cell development. B6 wild-type mice were included as a control. (A) Schematic representation of the MOT1 retrovirus (MSCV derived retrovirus expressing OT1 TCR cDNAs). MSCV: murine stem cell virus; LTR: long terminal repeat; IRES: internal ribosomal entry site; WRE: woodchuck responsive element. (B) Detection of HSCs expressing the OT1 TCR transgenes in bone marrow (BM) of RAG/MOT1 and B6/MOT1 host mice, by intracellular staining of TCR Vα2 and Vβ5.1,5.2. (C) Thymic development of OT1 T cells in RAG/MOT1 and B6/MOT1 host mice. Thymocytes expressing OT1 TCR transgenes were detected in both RAG/MOT1 and B6/MOT1 host mice by intracellular TCR staining (upper panel). The distribution of CD4 and CD8 expression on thymocytes is shown (lower panel). (D) Detection of mature OT1 CD8 T cells in the spleen of RAG/MOT1 and B6/MOT1 mice by FACS staining.

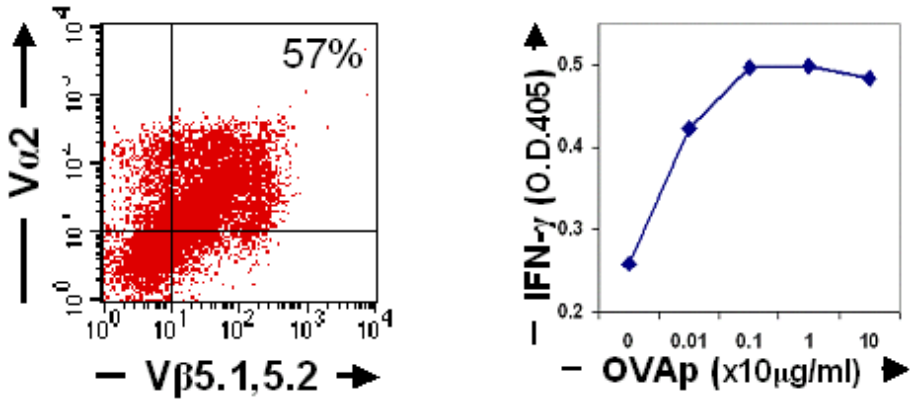

Figure 3-2: Functional expression of the OT1 TCR in primary CD8 T cells using MOT1 retroviruses. Primary CD4 T cells were harvested from B6 mice spleen and were stimulated *in vitro* with 0.5μg/ml anti-CD3 + 0.5μg/ml anti-CD28 for 3 days. On day2, the cells were spin-infected with MOT1 retroviruses. On day3, a fraction of cells was analyzed for surface expression of mouse TCR Vα2 and Vβ5.1,5.2 (the Vα and Vβ elements used by OT1 TCR) by FACS. As shown in the left panel, 57% of the CD8 T cells expressed OT1 TCR. The left cells were rested with 10ng/ml rmIL-2 overnight and then tested for their response to OVAp stimulation. Right panel showed the infected cells responded to OVAp stimulation measured by IFN-γ production using ELISA.

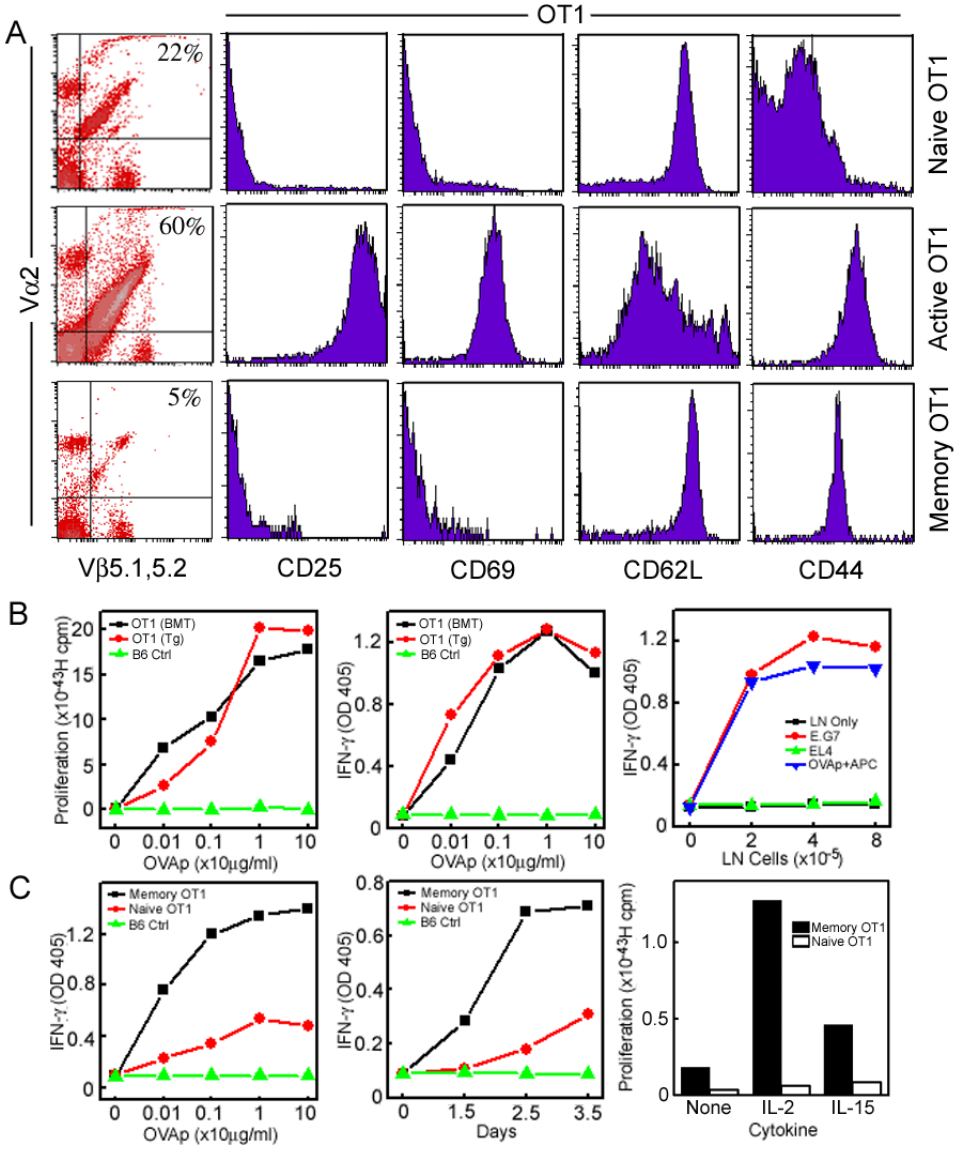
A
OT1
22%
60%
5%
Vα2
Naive OT1
Active OT1
Memory OT1
Vβ5.1,5.2
CD25
CD69
CD62L
CD44
B
OT1 (BMT)
OT1 (Tg)
B6 Ctrl
Proliferation (x10-4 3H cpm)
OVAp (x10μg/ml)
IFN-γ (OD 405)
LN Only
E.G7
EL4
OVAp+APC
LN Cells (x10-5)
C
Memory OT1
Naive OT1
B6 Ctrl
Days
None
IL-2
IL-15
Cytokine

Figure 3-3

Figure 3-3: Characterization of the OT1 T cells generated by viral transduction of wild-type B6 HSCs. OT1 T cells harvested from B6/MOT1 host mice 7 weeks after BM transfer were considered to be naïve. They were stimulated with OVAp *in vitro* for 3 days to generate active OT1 T cells, which were then transferred into $RAG1^{-/-}$ recipient mice. Sixteen weeks later, the recipient mice were analyzed for the presence of memory OT1 T cells. (A) Patterns of surface activation markers on OT1 T cells at the naïve, active or memory stages measured by FACS staining. Surface markers studied are indicated below each column of results. (B) Functional analysis of the naïve OT1 T cells. Proliferation (left) and IFN-γ production (middle) in response to OVAp stimulation, and IFN-γ production in response to E.G7 tumor cells (right) are shown. The responses were compared with those of conventional transgenic OT1 T cells. B6 spleen cells were included as a negative control. OT1 T cells generated from virus-transduced BM cells were designated as OT1(BMT), while the conventional transgenic OT1 T cells were designated as OT1(Tg). (C) Functional analysis of memory OT1 T cells. Dosage response (left) and time-course response (middle) to OVAp stimulation, and proliferation response to cytokine stimulation (right) are shown. The responses were compared with those of the naïve OT1 T cells. B6 spleen cells were included as a negative control.

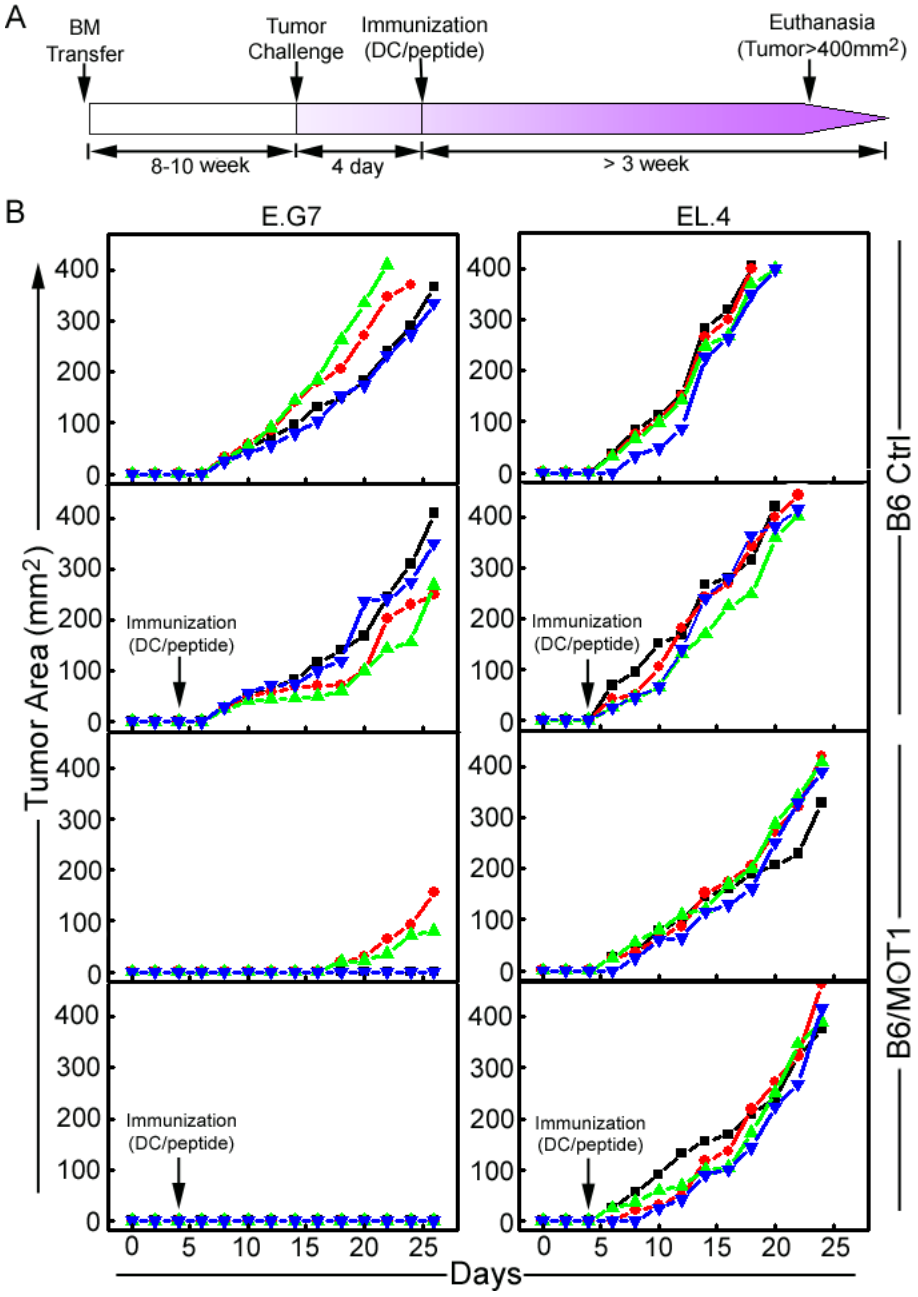
A
BM Transfer
Tumor Challenge
Immunization (DC/peptide)
Euthanasia (Tumor>400mm2)
8-10 week
4 day
> 3 week
B
E.G7
EL.4
Tumor Area (mm2)
Immunization (DC/peptide)
B6 Ctrl
B6/MOT1
Days

Figure 3-4

Figure 3-4: Suppression of syngenic tumor growth by imparting anti-tumor specificity to the mouse T cell repertoire via viral tranduction of HSCs. EL.4 and its Ova-expressing derivative, E.G7. were the two mouse tumor types examined. (A) Protocol for the tumorogenesis experiment. (B) Solid tumor growth in mice receiving different treatments. Tumor size is shown as the product of the two largest perpendicular diameters a x b (mm^2). Mice were euthanized when the tumors reached 400mm^2.

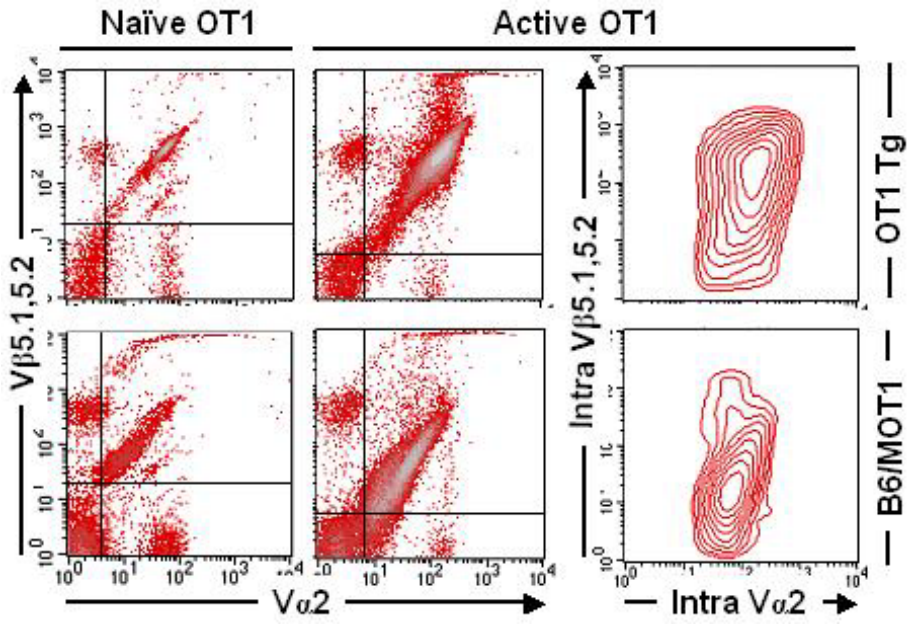
Naïve OT1
Active OT1
Vβ5.1,5.2
Vα2
Intra Vβ5.1,5.2
Intra Vα2
OT1 Tg
B6/MOT1

Figure 3-5

Figure 3-5: Comparison of TCR expression in OT1 T cells from B6/MOT1 mice (B6 hosts receiving MOT1 transduced B6 hematopoietic stem cells) with those from OT1 Tg mice (conventional OT1 TCR transgenic mice). Primary CD8 T cells were harvested from B6/MOT1 or OT1 Tg mice and stimulated with OVAp *in vitro*. The surface expression of OT1 TCRs (identified by co-staining of mouse TCR mouse TCR Vα2 and Vβ5.1,5.2) on naïve and activated OT1 T cells and the intracellular expression of OT1 TCR in activated OT1 T cells were analyzed by FACS. As shown, OT1 TCR expression in OT1 T cells from B6/MOT1 mice (mediated by retroviral LTR) is lower than that in OT1 T cells from OT1 Tg mice (mediated by endogenous TCR promoter). But the lower level of TCR does not affect the T cells' ability to respond to antigen. No obvious advantage has been observed for T cells expressing a higher level of TCR.

5. References and Notes

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