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Antibody treatment of human tumor xenografts elicits active anti-tumor immunity in nude mice

Meredith A. Liebman^a, Marly I. Roche^b, Brent R. Williams^a, Jae Kim^a, Steven C. Pageau^a, and Jacqueline Sharon^{a,*}

^a Department of Pathology and Laboratory Medicine, and the Hubert H. Humphrey Cancer Research Center, Boston University School of Medicine, Boston, MA 02118, USA

^b Department of Microbiology, Boston University School of Medicine, Boston, MA 02118, USA

Abstract

Athymic nude mice bearing subcutaneous tumor xenografts of the human anti-colorectal cancer cell line SW480 were used as a preclinical model to explore anti-tumor immunotherapies. Intratumor or systemic treatment of the mice with murine anti-SW480 serum, recombinant anti-SW480 polyclonal antibodies, or the anti-colorectal cancer monoclonal antibody CO17-1A, caused retardation or regression of SW480 tumor xenografts. Interestingly, when mice that had regressed their tumors were re-challenged with SW480 cells, these mice regressed the new tumors without further antibody treatment. Adoptive transfer of spleen cells from mice that had regressed their tumors conferred anti-tumor immunity to naïve nude mice. Pilot experiments suggest that the transferred anti-tumor immunity is mediated by T cells of both $\gamma\delta$ and $\alpha\beta$ lineages. These results demonstrate that passive anti-tumor immunotherapy can elicit active immunity and support a role for extra-thymic $\gamma\delta$ and $\alpha\beta$ T cells in tumor rejection. Implications for potential immunotherapies include injection of tumor nodules in cancer patients with anti-tumor antibodies to induce anti-tumor T cell immunity.

Keywords

Nude mice; T Cells; antibodies; tumor immunity; SW480 human tumor cell line; spleen cells; adoptive transfer

1. Introduction

$\gamma\delta$ as well as $\alpha\beta$ T cells are present in nu/nu (nude) mice despite the congenital absence of a thymus. The number of T cells in the spleen and lymph nodes of nude mice increases with age but it is still 5–10 times lower than the number of T cells in normal mice [1–4]. The level of IL-2, the T cell growth factor, also increases with age in nude mice [5]. Although only 2–4% of splenic T cells express $\gamma\delta$ TCRs in normal mice, as many as 50% of splenic T cells express $\gamma\delta$ TCRs in older nude mice [1,4]. Concurrent with the higher number of T cells in general and of $\gamma\delta$ T cells in particular, an age-related decrease in transplantability of human tumor xenografts into BALB/c nude mice has been found [5]. Furthermore, peritumoral injection of IL-2 into young adult BALB/c nude mice was found to inhibit growth of HeLa and Hu 609T human carcinoma xenografts [5], suggesting that T cells are responsible for the growth

* Corresponding author. Tel.: +1 617 638 4652; fax: +1 617 638 4079. E-mail address: jsharon@bu.edu (J. Sharon)..

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inhibition. Additional support for the involvement of T cells in tumor rejection in older nude mice came from the observation that when murine EL-4 lymphoma cells, transduced with the murine B7-1 gene, were injected into allogeneic BALB/c nude or SCID mice, tumors grew for several weeks and then regressed in BALB/c nude mice but grew without regression in SCID mice [6]. Tumor regression was prevented in 90% of these nude mice by treatment with anti-TCR $\gamma\delta$ antibodies but in only 50% of nude mice by treatment with anti-TCR $\alpha\beta$ antibodies, indicating a more important role for $\gamma\delta$ than for $\alpha\beta$ T cells in tumor rejection in nude mice. Similarly, whereas immunization of BALB/c nude mice with the B7-1 transduced EL-4 cells prevented the growth of wild type EL-4 cells, this protective immunity was abrogated by treatment with anti-TCR $\gamma\delta$ antibodies [6].

Nude mice have been used to show regression of human tumor xenografts in preclinical studies of anti-tumor antibodies [7–9]. In the current study, we show that such antibody-mediated regression of human tumor xenografts in BALB/c nude mice elicits active anti-tumor immunity, which can be transferred by spleen cells to naïve BALB/c nude mice. We also provide preliminary data suggesting that $\gamma\delta$ and $\alpha\beta$ T cells play the main role in this immunity.

2. Materials and Methods

2.1. Mice

Female 6–8 week old BALB/c nu/nu (BALB/c nude) and BALB/c mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA). All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Boston University Medical Campus.

2.2. Mammalian cells and cell culture

The human colorectal cancer cell line SW480 was obtained from the American Type Culture Collection (ATCC, Rockville, MD), and cultured at 37°C in L-15 medium supplemented with 10% (v/v) FBS. The CO17-1A murine hybridoma cell line, which produces a monoclonal antibody specific for the human tumor associated antigen EpCam (epithelial cell adhesion molecule) (107, 108), was obtained from Dr. Dorothee Herlyn of the Wistar Institute (Philadelphia, PA) and cultured at 37°C in a humidified environment of 5% CO₂/95% air in IMDM (containing 3.7 g NaHCO₃) supplemented with 5 µg/ml gentamicin and 10% FBS. Lib-Col2.1, a mixture of cell lines produced by transfection of vectors encoding murine recombinant polyclonal antibodies into Sp2/0 murine myeloma cells [10] was previously described [11]. Lib-Col2.1 cells were cultured under the same conditions as the CO17-1A hybridoma cells, except that the medium was supplemented with hypoxanthine, mycophenolic acid, and xanthine at 15, 6, and 250 µg/ml, respectively.

For mass culture, Lib-Col2.1 and CO17-1A cells were separately grown in spinner flasks in IMDM containing 3 g NaHCO₃ per liter and supplemented with 5 µg/ml gentamicin and 7% FBS, at 37°C; or in a Hollow Fiber Bioreactor (Fiber Cell Systems, Inc, Frederick, MD) in HYQSF4M4MAb utility medium (Hyclone, Logan, Utah) supplemented initially with 5% FBS, 5% conditioned medium (supernatant of the same cells cultured for routine maintenance) and 5 µg/ml gentamicin, which was gradually changed to HYQSF4M4MAb utility medium supplemented with 1% FBS and 5 µg/ml gentamicin. CO17-1A was also cultured in a two-compartment bioreactor CELLline (Integra Biosciences, Switzerland) in the same conditions used for the Hollow Fiber Bioreactor.

2.3. Preparation of anti-SW480 serum and purified antibody

To obtain anti-SW480 serum, BALB/c mice were immunized i.p. with 1×10^7 SW480 cells in PBS/CFA and boosted with SW480 cells in PBS/IFA or PBS. Antisera from different mice

were pooled and tested for reactivity to SW480 cells by ELISA as previously described [12]. Anti-SW480 titers ranged from 8,100 to 24,300. The immune serum (as well as non-immune serum used as control) were heat-treated at 56°C for 30 min to destroy complement.

Lib-Col2.1 and CO17-1A antibodies were purified from mass culture supernatants by affinity chromatography on either anti-mouse IgG Sepharose (Sigma, St. Louis, MO) or rec-Protein A-Sepharose® 4B conjugate (Zymed, San Francisco, CA) according to the manufacturers' protocols. Antibody concentration was determined by OD₂₈₀, and purity was assessed by SDS-PAGE.

2.4. Human Tumor Xenograft Mouse Model

For tumor development, mice were injected s.c., in the right or left flank, with 2.4×10^6 or 1×10^7 SW480 cells in a total volume of 100 μ l in L-15 medium. Tumor treatment was done by either intratumor (i.t.) injection of 100 μ l of BALB/c anti-SW480 serum or i.p. injection of 0.1 mg of purified antibody. Tumors were measured with a micro-caliper and the tumor volume was calculated using the formula $\text{Volume} = \text{length} \times \text{width} \times \text{height}$.

2.5. Adoptive Transfer

Cells and serum for adoptive transfer were obtained from 6 to 17 mo old BALB/c nude donor mice and injected i.p. into 7 wk to 7 mo old BALB/c nude recipient mice. Blood was obtained from donor mice, before sacrifice, via the retroorbital sinus under anesthesia, clotted, centrifuged, and serum collected. After sacrifice, spleen cells and lymph node cells were obtained by tissue perfusion. Peritoneal lavage was done by post-mortem i.p. injection of 2 ml of IMDM followed by the removal of the medium through a hole made in the peritoneal lining. Cells were transferred to recipient mice directly, after erythrocyte lysis with 0.83% NH_4Cl , or after magnetic antibody cell sorting (MACS). T cells were separated using a MACS® Pan T isolation kit from Miltenyi Biotec (Auburn, CA), which yields negatively selected T cells and positively selected non-T cells. In some experiments, the selected T cells were then divided into two aliquots and used for negative selection of $\alpha\beta$ or $\gamma\delta$ T cells: $\alpha\beta$ T cells were negatively selected with PE-conjugated hamster anti-mouse $\gamma\delta$ TCR monoclonal antibody (clone GL3, BD Biosciences) and magnetic microbeads coupled to anti-PE antibody (Miltenyi Biotec); and $\gamma\delta$ T cells were negatively selected with PE-conjugated hamster anti-mouse TCR β chain monoclonal antibody (clone H57-597, BD Biosciences) and magnetic microbeads coupled to anti-PE antibody. Stained cells were separated on MACS LS separation columns according to the manufacturer's recommendations (Miltenyi Biotec Inc). For positive selection of $\gamma\delta$ or $\alpha\beta$ T cells, cells were eluted from the respective columns according to manufacturer's directions. Cells were resuspended in IMDM for injection into recipient mice.

2.6. Flow Cytometry

For flow cytometry, cells were aliquoted into Falcon 5 ml polystyrene round bottom tubes (12 mm $\times$ 75 mm), at 1×10^6 cells per tube, and centrifuged at $450 \times g$ for 2 min at 4°C. The supernatants were vacuum aspirated and each cell pellet was resuspended in 50 μ l of BALB/c mouse serum diluted 1:50 in PBS/2% FBS. Following 15 min incubation on ice, either 1 μ l or 2 μ l of fluorescently labeled antibodies were added to each sample for either single or double color staining respectively. PE or FITC-conjugated hamster anti-mouse β chain monoclonal antibody (clone H57-597, BD Biosciences) was used to stain $\alpha\beta$ T cells, and PE or FITC-conjugated hamster anti-mouse $\gamma\delta$ TCR monoclonal antibody (clone GL3, BD Biosciences) was used to stain $\gamma\delta$ T cells. For the negative control, no fluorescently labeled antibody was added. Following 45 min incubation on ice with occasional mixing, 1 ml of cold PBS/2% FBS was added to each tube, mixed, and the tubes centrifuged at $450 \times g$ for 2 min at 4°C. The cells were washed twice more with cold PBS/2% FBS, then fixed with 1 ml per tube of PBS/2% formaldehyde and stored at 4°C in the dark until analysis. Fluorescence intensity was measured

using a Becton-Dickinson FACScan flow cytometer. Live lymphocytes were gated based on side and forward scatter parameters. Compensation for FL-1 and FL-2 was performed using single stained samples and data were analyzed using CELL Quest 3.3 software for analysis (BD Biosciences).

3. Results

3.1. Intratumor treatment with anti-SW480 serum causes retardation or regression of SW480 tumor xenografts in nude mice

The current project was initiated to explore the potential of recombinant polyclonal antibodies for cancer therapy. As proof of concept, BALB/c nude mice were injected subcutaneously (s.c.) with the human colon cancer cell line SW480 which is tumorigenic in 100% of nude mice when 1×10^7 cells per mouse are injected [13] (and our own observation). Once tumors developed, the mice were treated by intratumor (i.t.) injections of BALB/c anti-SW480 serum (immune serum) or BALB/c non-immune serum as control. As shown in Fig. 1, the immune serum caused retardation or regression of SW480 tumor xenografts. On sacrifice, the mean tumor volumes for mice treated with immune and non-immune sera were 179 mm³ and 836 mm³ respectively, a difference of 79% ($p = 0.017$). The difference in mean tumor weight between the two groups of mice (73%) correlated well with the difference in mean tumor volume.

3.2. Nude mice treated by intratumor injection of anti-SW480 serum show regression of both new and any remaining original tumor on re-challenge with SW480 cells

When SW480-injected/immune serum-treated BALB/c nude mice were re-challenged with SW480 cells (without further antiserum treatment), they showed regression of the new tumor as well as any remaining tumor from the original injection of SW480 cells. In contrast, age-matched naïve BALB/c nude mice that had neither received the original SW480 injection nor been treated with immune serum, but received SW480 cells at the time of re-challenge, developed tumors as expected (see Fig. 2). Because sera from mice that regressed the new tumor did not show reactivity to SW480 cells (data not shown), tumor regression was unlikely to be due to anti-tumor cell antibodies remaining from the original antiserum treatment. Rather, these results suggested that the antiserum treatment of the tumors elicits active anti-tumor immunity.

3.3. Nude mice treated by intraperitoneal injection of purified anti-colorectal cancer antibodies show regression of both new and any remaining original tumor on re-challenge with SW480 cells

To confirm that retardation or regression of the original tumor was caused by anti-tumor antibodies in the antiserum, BALB/c nude mice injected s.c. with SW480 cells were treated by i.p. injections of purified recombinant anti-SW480 polyclonal antibodies (previously described [11]) or the anti-colorectal cancer monoclonal antibody CO17-1A [7,14] and re-challenge with SW480 cells. As shown in Fig. 3, antibody treatment of mice caused retardation or regression of the original tumor and regression of both the new tumor and the original tumor, after re-challenge with SW480 cells; no tumor regression was seen in naïve BALB/c nude mice injected with SW480 cells at the time of re-challenge (Fig. 3). These results further supported the conclusion that antibody-mediated tumor regression elicits active anti-tumor immunity and showed that this immunity can be induced by systemic (i.p.) antibody treatment, not just by i.t. treatment. All subsequent experiments used the recombinant polyclonal antibodies or the CO17-1A monoclonal antibody to affect tumor regression by i.p. and/or i.t. injection.

3.4. Adoptive transfer of spleen cells from antibody-treated/SW480-immune nude mice causes tumor regression in naïve nude mice

To determine whether the anti-tumor immunity could be transferred to naïve BALB/c nude mice, we initially tested a mixture of spleen cells, peritoneal lavage cells, and serum – to increase our chances of seeing “an effect” in case the immunity involved multiple components. Because of the large range of SW480 tumor xenograft sizes in nude mice, despite the 100% tumorigenicity of 1×10^7 SW480 cells (see Fig. 1), only complete tumor regression was considered “an effect”. As shown in Fig. 4, naïve mice were able to regress completely SW480 tumor xenografts if they received cells and serum from antibody-treated/SW480-immune donors (mice that had been treated with antibody to regress their original SW480 tumor and then showed regression of a second SW480 tumor). In contrast, naïve mice that received cells and serum from naïve donors or from untreated donors (mice that had been injected with SW480 cells once but had not been treated with antibody to regress their tumor) were ineffective (Fig. 4).

Additional experiments showed that spleen cells alone or (erythrocyte-depleted) splenocytes were sufficient for transfer of anti-tumor immunity (Fig. 5). Furthermore, preliminary pilot experiments suggested that immunity could be transferred by isolated splenic T cells but not by non-T cells (Fig. 6), and by both $\gamma\delta$ and $\alpha\beta$ T cells (Fig. 7). The experiments with isolated $\gamma\delta$ and $\alpha\beta$ T cells were hampered by the variability in the number of $\alpha\beta$ versus $\gamma\delta$ cells in different nude mice and the inconsistency of cell separation, especially in obtaining a $\gamma\delta$ -enriched cell population. An initial experiment, in which only negatively selected $\gamma\delta$ or $\alpha\beta$ cells were used, showed that the $\gamma\delta$ -enriched cell population caused faster tumor regression than the $\alpha\beta$ -enriched cell population; although both the $\gamma\delta$ -enriched and the $\alpha\beta$ -enriched cell populations affected complete tumor regression in the recipient mice (see Fig. 7A). However, in another experiment, in which both positively and negatively selected cell populations were used, in order to obtain sufficient numbers of cells, only the $\alpha\beta$ -enriched cell population caused complete tumor regression, whereas the $\gamma\delta$ -enriched cell population caused only partial tumor regression (Fig. 7B).

4. Discussion

We showed that intratumor treatment of SW480 human tumor xenografts with murine anti-SW480 immune serum causes retardation or regression of tumor growth in BALB/c nude mice (Fig. 1) and induces regression of both new and any remaining original tumor when mice are re-challenged with SW480 cells without additional immune serum treatment (Fig. 2). The effect of the immune serum is due to antibodies because it could be reproduced with purified SW480-reactive polyclonal or monoclonal antibodies (Fig. 3). The rejection of new and remaining original tumor is likely mediated by newly activated and/or expanded $\gamma\delta$ and/or $\alpha\beta$ T cells because the ability to reject SW480 tumors could be transferred to naïve BALB/c nude mice with T cells or $\gamma\delta$ - and/or $\alpha\beta$ -enriched T cell populations in preliminary pilot experiments (Figs. 6 and 7). The inconsistency as to which T cell population, $\gamma\delta$ or $\alpha\beta$, is more effective at adoptive transfer of protective anti-tumor immunity in the two pilot experiments (Fig. 7A and 7B) is probably a reflection of the low number of total T cells present in nude mice, where only an oligoclonal population is likely stimulated in each mouse, in a stochastic process. Studies with more mice are needed for firmer conclusions.

To interpret the ability of passive (antibody-mediated) immunotherapy of tumors to elicit active (likely T cell-mediated) immunity, we assume that the anti-SW480 antibodies used for treatment attract inflammatory cells leading to destruction of the tumor cells. Apoptosis or necrosis of the tumor cells may then activate anti-tumor T cells. Alternatively or in addition, the killing of tumor cells through antibody-mediated effector functions may allow sufficient time for T cell immunity to develop before the tumor overwhelms the immune system.

It is noteworthy that protective immunity against SW480 cells, both in donor and recipient mice, manifests as initial tumor growth followed by tumor regression rather than outright prevention of tumor growth (Figs. 2–7). It is also noteworthy that in some donor mice, in which antibody treatment has not completely regressed the original tumor, regression of the second tumor leads to complete or partial regression of the original tumor. Thus, in Fig. 2A, one of the two original tumors that had not been regressed at the time of second tumor cell injection (day 33) was completely regressed by day 52 (the other original tumor continued to grow and the mouse was sacrificed). Similarly, in Fig. 3A, all three of the original tumors that had not been regressed at the time of second tumor cell injection (day 56) were completely or almost completely regressed by day 85. Taken together, these observations suggest that activation and/or expansion of anti-tumor T cells requires the *in vivo* growth of the injected SW480 cells (as re-challenge in donor mice or first challenge in recipient mice).

Because nude mice have no thymus, the development of T cell mediated anti-tumor immunity implies that extra-thymic T cells are being stimulated and expanded. Such extra-thymic T cells may be important in anti-tumor protection, especially against epithelial cancer cells in subcutaneous or intradermal sites. These cells may not be as evident in immunocompetent hosts due to either suppression by regulatory T cells and/or the unavailability of “space” for adequate expansion.

Studies by others have shown that if immunocompetent (C57BL/6) mice transplanted with syngeneic EL4 lymphoma cells are cured of their tumor by a chemotherapy-cytokine combination –they develop life-long immunity and reject re-implanted EL4 lymphomas [15–18]. The chemotherapy-cytokine treatments included cyclophosphamide plus TNF- α [15,16] [18] and doxorubicin plus IL-2 [17]. Treatment with doxorubicin plus TNF- α , which results in prolonged survival but no cure, also gave rise to T cell-mediated immunity [19,20], including rejection of implants of doxorubicin-resistant EL4 cells [19]. This immunity was correlated with the presence of anti-EL4 CD8⁺CD44⁺ CTLs [16–18,20], but no adoptive transfer experiments were reported. Interestingly, it was noted that in C57BL/6 mice that received a curative cyclophosphamide plus TNF- α combination, there was initially thymic involution followed by regrowth [15,18]. This phenomenon may mirror the situation in nude mice where “space” for expansion of anti-tumor T cell clones is available due the paucity of T cells.

The C57BL/6-EL4 studies, like our studies, also suggest that *in vivo* growth and/or killing of tumor cells may be necessary for development of active anti-tumor immunity. Moreover, because the EL4 lymphoma cells are syngeneic in C57BL/6 mice, the induced anti-EL4 immunity is unlikely to be directed against MHC antigens. Similarly, we believe that the induced anti-SW480 immunity in nude mice is not primarily due to mouse T cells cross-reacting with human MHC molecules; because if this were the case, then tumor xenografts could not form at all, as seen in immunocompetent mice, in which most T cells are educated in the thymus to recognize MHC molecules. Therefore, the induced anti-SW480 immunity is likely directed against other xenogeneic antigens which may or may not be presented by MHC molecules.

The ability of immune serum or antibodies to induce active anti-tumor immunity in nude mice could potentially be harnessed for the development of cancer immunotherapies in humans. One possibility would be the injection of tumor nodules, in cancer patients, with anti-tumor antibodies, to induce active anti-tumor immunity. Another possibility would be to clone TCRs from anti-human tumor T cells developed in nude mice and construct vectors encoding chimeric or humanized TCRs. Such vectors could be transfected or transduced into autologous T cells for adoptive transfer into cancer patients.

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Fig. 1. Intratumor treatment with BALB/c anti-SW480 immune serum causes retardation or regression of SW480 tumor xenografts. BALB/c nude mice (6 per group, 8 wk old) were injected s.c. in the right flank with 1×10^7 SW480 cells, and received i.t. injections of heat-treated (56°C 30 min) BALB/c serum beginning 3–5 days later (once a measurable tumor developed). One group received BALB/c anti-SW480 immune serum and the other group received BALB/c non-immune serum. All mice were sacrificed and tumors resected and weighed on day 14. A) Tumor volumes. Each curve represents an individual mouse. Arrows indicate time points of serum injections. B) Tumor weights at sacrifice.

Fig. 2.

Mice treated by intratumor injection of anti-SW480 serum show regression of both new and any remaining original tumor on re-challenge with SW480 cells. A) BALB/c nude mice were injected s.c. with 1×10^7 SW480 cells in the right flank on day 0 and treated with 12 i.t. injections of BALB/c anti-SW480 immune serum between days 3 and 23, then re-challenged by s.c. injection of 1×10^7 SW480 cells in the left flank on day 33 (arrow). B) Naïve BALB/c nude mice, sex- and age-matched to the mice in A, were injected with 1×10^7 SW480 cells in the left flank on the same day as the mice in A. Each bar represents the tumor volume in an individual mouse on a given day; mice are shown in the same order each day. Bars below the X axis indicate “no detectable tumor”.

Fig. 3.

Mice treated by intraperitoneal injection of purified anti-colorectal cancer antibodies show regression of both new and any remaining original tumor on re-challenge with SW480 cells. A) BALB/c nude mice were injected s.c. with 2.4×10^6 SW480 cells in the right flank on day 0 followed, within one hour, by i.p. injection of 100 μ g of purified anti-SW480 recombinant antibodies or the anti-colorectal cancer MAb CO17-1A; the i.p. injections, with the respective antibody preparations, were repeated on days 1, 2, 3, 4, 5, 8, 10, 12 and 14. Mice were re-challenged by s.c. injection of 1×10^7 SW480 cells in the left flank on day 56 (arrow). B) Naïve BALB/c nude mice were treated only by s.c. injection of 1×10^7 SW480 cells in the left flank on day 56. Each bar represents the tumor volume in an individual mouse on a given day; mice are shown in the same order each day. Bars below the X axis indicate “no detectable tumor”. Note change of scale for day 85.

Mice received cells and serum from the following donors:

Fig. 4.

Adoptive transfer of cells and serum from antibody-treated/SW480-immune mice causes tumor regression in recipient mice. Naïve BALB/c nude mice (three per group) were injected s.c. with 1×10^7 SW480 cells on day 0. The mice were then injected i.p. with pooled spleen and peritoneal lavage cells from BALB/c nude donor mice, on days 2 and 5 and with 0.1 ml of serum from the same donors on days 3 and 6. A total of four or five donors was used for each group of recipient mice. “Untreated donors” had been injected with SW480 cells once but had not been treated with antibody to regress their tumor. Each curve represents tumor volumes in one recipient mouse.

Fig. 5.

Adoptive transfer of splenocytes from antibody-treated/SW480-immune mice causes tumor regression in recipient mice. Nine naïve BALB/c nude mice were injected s.c. with 1×10^7 SW480 cells on day 0. Seven of the mice were then injected i.p. with pooled cells from BALB/c nude donor mice, on days 2 and 5 or with 0.1 ml of serum from the same donors on days 3 and 6. A total of four donors was used for adoptive transfer into seven recipient mice. Two of the nine mice were used as control and received neither cells nor serum ("None"). Each curve represents tumor volumes in one recipient mouse.

Fig. 6.

Adoptive transfer of splenic T cells from antibody-treated/SW480-immune mice is sufficient to cause tumor regression in recipient mice. Two naïve BALB/c nude mice were injected s.c. in the right flank with 1×10^7 SW480 cells on day 0. On days 2 and 5 post tumor cell injection, the mice were injected i.p. with cells from SW480-immune donor mice. The donor cells were prepared on each day 2 and 5 from the spleen of one donor. One mouse received the T cells (2.2×10^7 and 3.0×10^7 cells on days 2 and 5 respectively), and the other mouse received the non-T cells (4.7×10^7 and 2.5×10^8 cells on days 2 and 5 respectively). A) Flow cytometry analysis of the T cell and non-T cell populations (shown for day 5). B) Tumor volumes in recipient mice.

Fig. 7. Adoptive transfer of $\gamma\delta$ and/or $\alpha\beta$ T cells from antibody-treated/SW480-immune nude mice causes tumor regression in naïve nude mice. A) Two naïve BALB/c nude mice were injected s.c. in the right flank with 1×10^7 SW480 cells on day 0. On day 1 post tumor cell injection, the mice were injected i.p. with cells from SW480-immune donor mice. To prepare the donor cells, spleen cells from two donors were pooled, T cells were isolated and $\alpha\beta$ and $\gamma\delta$ T cells were negatively-selected. One mouse received the $\alpha\beta$ T cells (6.7×10^6 cells) and the other mouse received the $\gamma\delta$ T cells (2.7×10^6 cells). B) Two naïve BALB/c nude mice were injected s.c. in the right flank with 1×10^7 SW480 cells on day 0. On days 2 and 5 post tumor cell injection, the mice were injected i.p. with cells from SW480-immune donor mice. To prepare the donor

cells on each day 2 or 5, spleen cells and lymph node cells from two donors were pooled, T cells were isolated and $\alpha\beta$ and $\gamma\delta$ T cells were both positively and negatively-selected. One mouse received all the $\alpha\beta$ T cells (1.4×10^6 and 7.6×10^6 on days 2 and 5 respectively) and the other mouse received all the $\gamma\delta$ T cells (1.1×10^6 and 4.3×10^6 on days 2 and 5 respectively).