

Eradication of Disseminated Lymphomas with CpG-DNA Activated T Helper Type 1 Cells from Nontransgenic Mice¹

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Abstract

Various evidence suggests that adoptive transfer of polyclonal, tumor-specific, IFN- γ -producing CD4⁺ T cells [T helper type 1 (Th1) cells] should be highly efficient for tumor immune therapy. However, this approach could not be tested because very few MHC class II-restricted tumor peptides have been defined. Here we show that stimulation of freshly isolated T helper cells with syngeneic tumor cells and antigen-presenting cells in the presence of immunostimulatory CpG DNA allows the generation of large numbers of strongly polarized, tumor-specific Th1 cells within 3 weeks of culture, even when T helper cells were derived from tumor-bearing mice. A single injection of 0.5×10^6 A20-specific Th1 cells even eradicated disseminated A20 lymphomas and provided lifelong protection without inducing autoimmune disease. The therapy was largely independent of CD8⁺ cells but required IFN- γ and CD40-CD40L interactions, suggesting that tumor-specific Th1 cells eradicate established tumors by activating proinflammatory macrophages.

Introduction

Active immunization with tumor antigens may induce inflammatory “autoimmune” responses against MHC class I-restricted tumor epitopes and protect against a large variety of tumors in mice and probably also in humans (1–3). Active immunization can even be used for tumor therapy, but the therapeutic efficacy is still limited. Loading dendritic APCs⁴ with total tumor extracts or peptides, modifying tumor cells with cytokines or costimulatory molecules, or stimulating T cells through CD28/CTLA-4 enhances the efficiency of antitumor vaccines but increases the risk of inducing autoimmune disease (1–3).

Recent insights into the crucial role of CD4⁺ T cells in orchestrating antitumor immunity (4–7) and the power of adoptively transferred IFN- γ -producing Th1 cells in specifically destroying targeted tissues (8) suggest that adoptive transfer of tumor-specific Th1 cell lines could be a safe and effective approach to tumor therapy. However, only a few MHC class II-restricted tumor peptide epitopes have been characterized to date (9), a fact that has strongly impaired the exact analysis of tumor-specific CD4⁺ T cells, IFN- γ -producing Th1 cells, and IL-4-producing Th2 cells in tumor immunity. Generation of tumor-specific Th1 cell clones *in vitro* (7) or transfection of tumors with antigens recognized by T-cell receptor-transgenic lymphocytes from healthy mice (10) allowed to address some important questions,

but these systems did not analyze the therapeutic potential of naturally occurring, tumor-reactive Th1 and Th2 cells or the properties of tumor-specific T cells occurring in tumor-bearing animals.

Because immunostimulatory CpG ODNs promote maturation of APCs and induce the expression of costimulatory molecules and the production of IL-12 (11–13), they should be useful as an *in vitro* adjuvant for the rapid generation of tumor-specific Th1 cells. Here we report that immunostimulatory CpG ODN 1668 allowed us to generate large amounts of tumor-specific Th1 and Th2 cells within 3 weeks of culture, surprisingly even when CD4⁺ T cells were isolated from mice with advanced tumors. Adoptive transfer of these tumor-specific Th1 cells efficiently eradicated even established tumors in a CD40L- and IFN- γ -dependent fashion, whereas *in vitro*-generated, IL-4-producing Th2 cells prolonged survival but ultimately failed to cure animals.

Materials and Methods

Media and Reagents. A20 lymphoma cells, MPC11 plasmacytoma cells, and BiV trioma cells were cultured in RPMI 1640 supplemented with 5% FCS, 2-mercaptoethanol, and 2 mM glutamine (Biochrom, Berlin, Germany) at 37°C in a humidified atmosphere with 5% CO₂. BiV trioma cells are hybrids of the A20 lymphoma and the 2.4G2 hybridoma specific for the receptors Fc γ RII/III. Vaccination with BiV trioma cells that express the tumor idiotype as an immunoglobulin half molecule attached to the APC-binding arm confers efficient immunity against the wild-type tumor (14). T-cell lines were cultured in DMEM supplemented with 10% FCS and 2-mercaptoethanol. Phosphothioate-modified CpG ODN 1668 (5'-TCCATGACGTTCCTGATGCT-3') was from MWG (München, Germany). The IL-4 and IFN- γ content of culture supernatants was determined using commercially available ELISA Abs (PharMingen, Hamburg, Germany).

T-cell Lines. CD4⁺ T cells were derived from spleen and lymph node cells from mice immunized twice with 10^5 BiV trioma cells (14) or from mice with a large tumor burden (>day 30). These CD4⁺ T cells were enriched by negative selection over Biotex T-cell columns (TEBU, Frankfurt, Germany) to 95% purity. No CD8⁺ T cells were detectable by fluorescence-activated cell-sorting analysis. The CD4⁺ T cells (2×10^5) were stimulated *in vitro* in 96-well round-bottomed tissue culture plates in the presence of irradiated (30 Gy) T-cell-depleted syngeneic spleen cells, 3×10^5 irradiated (90 Gy; from a ¹³⁷Cs source) A20 tumor cells, CpG ODN 1668 (0.2 μ M), IL-2 (5 units/ml; a gift from Chiron, Ratingen, Germany), and anti-IL-4 mAb 11B11 (10 μ g/ml) to generate Th1 lines. To generate Th2 cell lines, IL-4 (1000 units/ml) was added to the culture instead of anti-IL-4 mAb. Recombinant IL-4 was derived from X63Ag-653 cells (a gift of F. Melchers; Basel Institute of Immunology, Basel, Switzerland). On day 10, cells were purified on a Ficoll gradient and restimulated as described on day 1. The cells were expanded for another 7 days, and on day 20, these >99% pure CD4⁺ T cells were used for adoptive transfer, and a fraction of these cells were used for functional characterization.

To generate anti-A20 CD8⁺ T cell lines, 4×10^6 spleen and lymph node cells from trioma-vaccinated mice were suspended in medium, seeded in 24-well plates, and mixed with 10^5 irradiated (100 Gy) A20 cells. A restimulation followed at day 6 with an additional 10^6 naive syngeneic spleen cells/well (irradiated at 30 Gy). On day 8, 30 units/ml IL-2 were added. One day later, T-cell reactivity against irradiated A20 or MPC11 cells was determined by [³H]thymidine incorporation or by measuring granulocyte macro-

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⁴ The abbreviations used are: APC, antigen-presenting cells; ODN, oligodeoxynucleotide; Th, T helper; IL, interleukin; mAb, monoclonal antibody; Ab, antibody; CD40L, CD40 ligand; GNA, concanavalin A; OVA, ovalbumine.

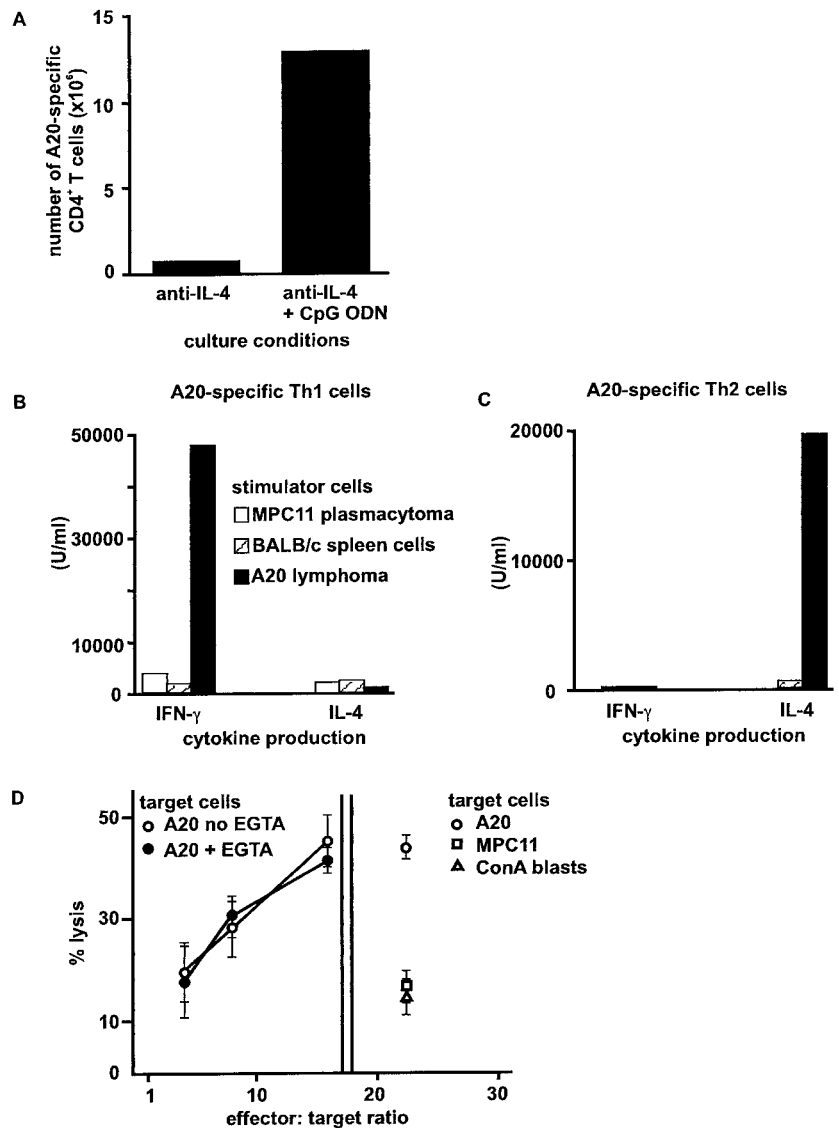


Fig. 1. Rapid *in vitro* generation of A20-specific Th1 cells using CpG ODNs. **A**, expansion of A20-specific Th1 cells stimulated in the presence of anti-IL-4 mAb 11B11 (*left*) or in the presence of anti-IL-4 mAb 11B11 and CpG ODN 1668 (*right*) derived from BALB/c mice that were immunized with BiV trioma cells (see "Materials and Methods"). Specificity and cytokine pattern of the Th1 cells are given in **B**. Cytokine production by A20-specific (**B**) Th1 and (**C**) Th2 cell lines derived from BALB/c mice immunized with BiV trioma cells. The Th1 and Th2 cell lines were generated as described in "Materials and Methods." For determination of the cytokine pattern, Th cell lines were stimulated overnight in the presence of APCs with or without irradiated A20 lymphoma or MPC11 plasmacytoma cells. The IL-4 and IFN- γ content in the 24-h culture supernatant was determined by ELISA. **D**, antigen-specific cytotoxicity and Ca²⁺ independence of A20-reactive Th1 cell lines. Th1 cells from BiV trioma-immunized BALB/c mice were generated as described in **A**, and the lysis of the different target cells was measured using the JAM test. Cytotoxicity tests were performed in the presence (*closed symbols*) or absence (*open symbols*) of 1 mM EGTA and 1.5 mM MgCl₂. Experiments were performed in triplicates. Two of three independent experiments are shown.

phage colony-stimulating factor secretion in an ELISA. Before *in vivo* application, the remaining CD4⁺ T cells were depleted by immunomagnetic separation. This population was >99% CD8⁺.

Animal Experiments. Mice were purchased from Bomimice (Ry, Denmark). All experiments were done with groups of five or six female animals. For immunization, mice were injected i.p. with 10⁵ BiV trioma cells twice in a 3-week interval (14). In the tumor prevention experiments shown, the animals received a lethal A20 tumor dose (3 × 10⁵ cells) together with 5 × 10⁵ CD4⁺ T cells i.p. To evaluate therapy of a preexisting tumor burden, mice were challenged first with a lethal i.v. A20 inoculum (3 × 10⁵ cells) and treated with 5 × 10⁵ Th1 cells i.v. on day 5 or 7. Mice were euthanized when signs of tumor growth became visible. CD8⁺ T cells were depleted *in vivo* by five i.p. injections of 0.5 mg of the anti-CD8 mAb RmCD8.2 (14) every 3–8 days starting 4 days before Th1 therapy. IFN- γ was neutralized by injecting 0.5 mg of XMG-1.2 mAb (15) i.p. 24 h and again 4 h before Th1 cell treatment. The anti-CD40L mAb MR-1 (16) was given at a dose of 0.25 mg i.p. on day 0, 2, 4, and 8 of Th1 therapy. All animal experiments were performed at least twice. Statistical survival analyses were done using the log-rank test.

ELISA. To evaluate the humoral response against A20 cells after Th1 therapy, mouse sera were tested for binding to purified A20 immunoglobulin immobilized on ELISA plates or to A20 cells in a cellular ELISA. Reactivity was detected by incubating these plates with a peroxidase-conjugated polyclonal goat antimouse IgM Ab or an anti-IgG Ab that had been adsorbed against mouse IgG2a. The IL-4 and IFN- γ content in the 24 h culture super-

natant of stimulated T cells was determined as described (12). All ELISA Abs were from PharMingen.

Cytotoxicity Assay. Fresh spleen cells (2 × 10⁶) were stimulated with ConA (2 μ g/ml) for 36 h to obtain ConA blasts. The ConA blasts, A20 lymphoma cells, and MPC11 plasmacytoma cells were labeled with [³H]thymidine (5 μ Ci/ml) for 8 h at 37°C. These target cells were washed twice with medium and then mixed (5 × 10⁴ cells/well) with the indicated numbers of effector cells in 96-well round-bottomed plates. After incubation for 2 h at 37°C, cells were harvested on glass fiber filters, and [³H]thymidine uptake was measured using an automatic filter counting system (TRACE96; Inotech, Dottikon, Switzerland). The percentage of specific lysis was calculated as described previously (17).

Results and Discussion

Rapid *in Vitro* Generation of Tumor-specific Th1 Cells Using CpG ODN 1668. To study the *in vitro* generation of tumor-specific Th1 cells and adoptive transfer of Th1 cells in antitumor immunity, we selected the poorly immunogenic A20 B-cell lymphoma, one of the most well-established lymphoma models in mice (14, 18). Highly purified CD4⁺ T cells were first derived from BALB/c mice immunized with a hybrid cell line of the A20 lymphoma and the 2.4G2 hybridoma (BiV trioma; Ref. 14) and were stimulated *in vitro* with

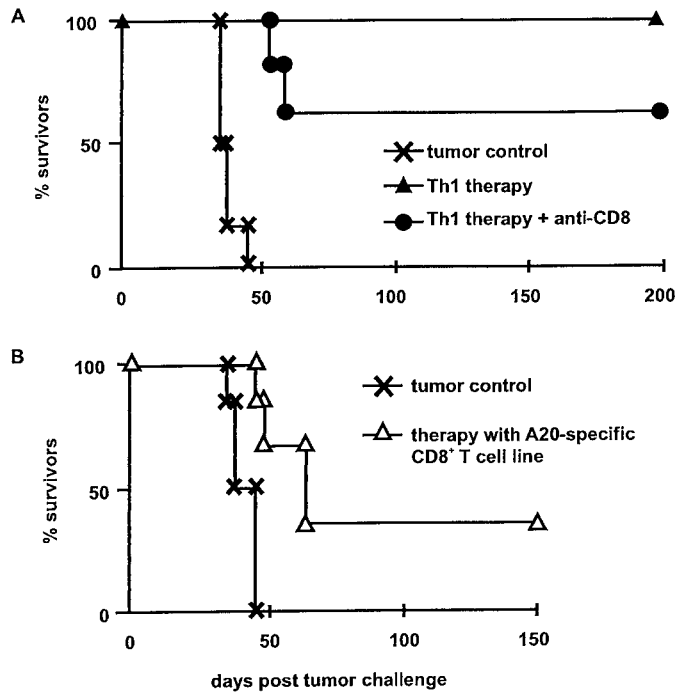


Fig. 2. *In vitro*-generated, A20-specific CD4⁺ Th1 cells provide better protection than A20-specific CD8⁺ cytotoxic T-cell lines. A, tumor protection by adoptive transfer of CD4⁺ Th1 cells. BALB/c mice received a lethal i.p. A20 cell dose (3×10^5 cells) and 5×10^5 A20-specific Th1 cells. The tumor control group received only 3×10^5 A20 cells. CD8⁺ T cells were depleted with mAb RmCD8.2 B, tumor protection by adoptive transfer of CD8⁺ T cell lines. BALB/c mice received a lethal i.p. A20 cell dose (3×10^5 cells) and 5×10^5 A20-specific CD8⁺ cytotoxic T cells. The tumor control group received only 3×10^5 A20 cells.

APCs and irradiated A20 lymphoma cells. Using the immunostimulatory CpG ODN 1668 as an *in vitro* adjuvant (12), this procedure provided $3\text{--}6 \times 10^6$ CD4⁺ T cells (>99% CD4⁺) per mouse within 3 weeks. Cultures initiated in the presence of CpG ODNs and anti-IL-4 mAb provided 12–70 times more CD4⁺ T cells than cultures initiated in the absence of CpG ODNs (Fig. 1A). These CD4⁺ T cells produced about 50 times more IFN- γ than IL-4 when exposed to A20 cells. No specific cytokine production was observed when the cells were exposed to syngeneic APCs or to an unrelated lymphoma, MPC11 (Fig. 1B). Because these CD4⁺ T cells specifically killed A20 lymphomas *in vitro* in a Ca²⁺-independent fashion (Fig. 1D), these cells were A20-specific Th1 cells.

Adoptive Transfer of A20-specific Th1 Cells Provides Protection against A20 Lymphomas. Adoptive transfer of 0.5×10^6 A20-specific Th1 cells (20×10^6 cells/kg bodyweight) protected 80–100% of mice against a simultaneous challenge with a lethal dose of i.p. or i.v. applied A20 lymphoma cells (Fig. 2A). Adoptive transfer of Th1 cells was more efficient than adoptive transfer of A20-specific CD8⁺ cytotoxic T-cell lines (Fig. 2B). Moreover, Th1 cells protected even CD8-depleted mice from the A20 lymphoma (Fig. 2A), suggesting that CD8-independent mechanisms play an important role in Th1 cell-mediated tumor protection. The treatment provided lifelong protection (up to 2 years), and no tumor cells were detected in spleen or bone marrow by fluorescence-activated cell-sorting analyses. More importantly, no dormant tumor cells were detectable in long-term cultures from spleen or bone marrow cells of Th1 cell-treated mice (data not shown).

The *in vitro* data (Fig. 1B) suggested that A20-reactive Th1 cells predominantly recognized tumor-associated antigens. Because adoptive transfer of autoreactive T cells can induce severe autoimmune disease (8, 13), and because the tumor-reactive Th1 lines generated *in*

vitro may recognize surface proteins shared between normal tissue and A20 cells, we performed extensive pathological examination of the Th1 cell-treated animals. Peripheral blood cell counts and histology of spleen, lymph nodes, bone marrow, liver, kidney, heart, lung, small bowel, and brain were all normal and showed no signs of autoimmune disease.

Adoptive Transfer of A20-specific Th1 Cells Eradicates Disseminated Lymphomas by a CD40L- and IFN- γ -dependent Mechanism. Adoptive transfer of A20-reactive Th1 cells was safe and effective in preventing tumors. Therefore, we analyzed the cells'

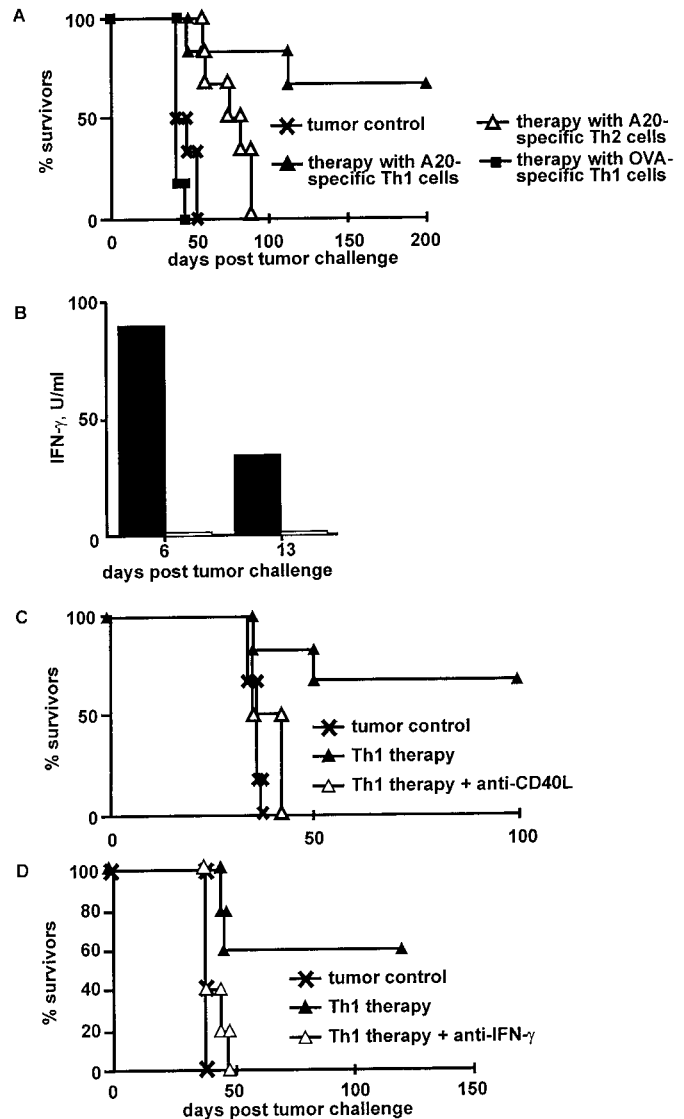


Fig. 3. Eradication of established A20 lymphomas by tumor-specific Th1 cells. A, survival of mice that received a lethal A20 challenge i.v. and then received a single injection of 5×10^5 T cells i.v. on day 7. The transferred T cells were either A20-specific Th1 cells, OVA-specific Th1 cells, or A20-specific Th2 cells. B, persistence of anti-A20-specific immune responses after adoptive transfer of A20-specific Th1 cells. BALB/c mice received a single i.p. injection of 3×10^5 A20 cells alone (□) or in combination with 0.5×10^6 Th1 cells (■). On day 6 and day 13, spleen cells were stimulated *in vitro* in the presence or absence of irradiated A20 cells. The IFN- γ content in the 24-h culture supernatant was determined by ELISA. One of two similar experiments is shown. C, Th1 cell therapy of established lymphomas depends on CD40-CD40L interactions. Mice were treated as described in the Fig. 3A legend. They received Th1 cells on day 5 after tumor application and received four i.p. injections of 0.25 mg of anti-CD40L antibody MR-1 on days 0, 2, 4, and 8 of Th1 cell therapy. D, tumor therapy with Th1 cells is abolished by neutralization of IFN- γ . Mice were treated as described in the Fig. 3A legend. In addition, they received two i.p. injections of 0.5 mg of the anti-IFN- γ mAb XMg-1.2 one day and 4 h before transfer of A20-specific Th1 cells (given 5 days after the A20 cells). Unrelated rat IgG1 mAb, rat IgG2b mAb, or hamster serum did not influence the Th1 therapy (data not shown).

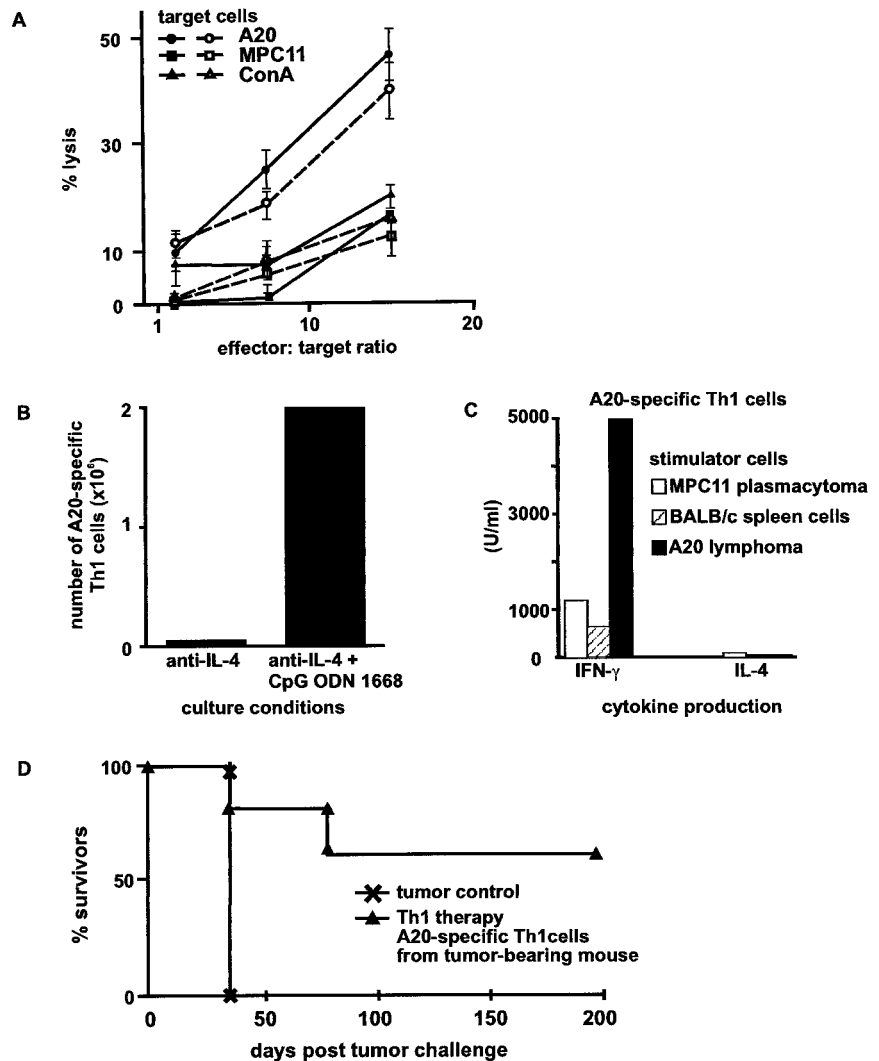


Fig. 4. Tumor protection with anti-A20-specific Th1 cells from tumor-bearing animals. **A**, cytolytic activity of CD4⁺ T cells from tumor-bearing (closed symbols) or BiV-immunized (open symbols) BALB/c mice. CD4⁺ T cells from mice with a large tumor burden (>day 30) were stimulated for 10 days with syngeneic irradiated APCs, irradiated A20 cells, CpG ODN 1668, IL-2, and 11B11 mAb. Lysis of A20 cells, MPC11 cells, or ConA blast target cells was determined using the JAM test. Mean values of triplicates are given. One of four representative experiments is shown. Similar results were obtained with total spleen cells. **B**, expansion of A20-specific Th1 cells from mice with a large tumor burden (>day 30). The CD4⁺ cells were stimulated in the presence of anti-IL-4 mAb 11B11 (left) or in the presence of anti-IL-4 mAb 11B11 and CpG ODN 1668 (right). Specificity and cytokine pattern of the Th1 cells are given in **C**. **C**, induction of IFN- γ -producing, A20-specific Th1 cell lines with CD4⁺ T cells derived from mice with a large tumor burden. Th1 cells were generated from BALB/c mice with progressive tumor disease as described in "Materials and Methods." Cells were stimulated overnight with irradiated APCs in the presence or absence of irradiated A20 or MPC11 tumor cells. The IFN- γ and IL-4 content in the 24-h culture supernatant was determined by ELISA. **D**, rejection of A20 tumor cells in mice by A20-specific Th1 cells derived from tumor-bearing animals. Mice were treated as described in the Fig. 2A legend but with Th1 from tumor-bearing mice.

capacity in the therapy of established tumors. Within 5 days, injection of 0.3×10^6 A20 lymphoma cells establishes a tumor burden that responds only to the most efficient immunization protocols (14, 18), and in the following 3 days, the splenic tumor burden increased again at least 100-fold (analyzed in four mice). When given 5 days or even 7 days after establishing the lymphoma, adoptive transfer of 0.5×10^6 A20-reactive Th1 cells still cured up to 70% of the animals (Fig. 3A). The therapy provided long-lasting immunity because 10 of 10 mice rejected a lethal A20 rechallenge on day 100 or 125. Effective therapy was dependent on Th1 cell recognition of tumor-associated peptides because identically generated, ovalbumine-specific Th1 cells provided no protection (Fig. 3A). Importantly, the therapy required a high IFN- γ :IL-4 ratio. Th0 cells producing both IL-4 and IFN- γ as well as IL-4-producing Th2 cells, which can induce strong immunoglobulin production and attract eosinophils (6, 7), significantly prolonged survival. However, all IL-4-producing Th0 and Th2 cell lines tested were unable to cure mice of the lymphoma (shown for Th2 cells in Figs. 1C and 3A). The high therapeutic efficacy of A20-reactive Th1 cells was surprising because it was shown that naive tumor-specific CD4⁺ T cells from transgenic animals are immediately silenced and provide no protection when transferred into tumor-bearing mice (10). In contrast, transfer of tumor-specific Th1 cell lines established persistent, A20-specific Th1 responses *in vivo* because spleen cells from Th1-treated mice produced large amounts of IFN- γ when stimulated

directly *ex vivo* with A20 cells (Fig. 3B). It is not known whether this IFN- γ was produced by CD4⁺ or CD8⁺ T cells and whether the responding Th1 cells are derived from the donor or the recipient. However, a series of unrelated experiments suggests that most of the IFN- γ is derived from the transferred Th1 cells.⁵

An increasing amount of data shows that CD4⁺ T cells play a pivotal role in orchestrating tumor immune responses. Their *in vivo* activity against MHC class II-positive and MHC class II-negative tumors has been associated with their capacity to provide help for CD8-mediated killing (19), induce IFN- γ -dependent monocyte/macrophage-mediated cytotoxicity (4, 7), or induce production of antitumor immunoglobulin by B cells (5). All three mechanisms are dependent on CD40-CD40L interactions (16, 20). To determine the contribution of CD40-CD40L interactions to Th1 cell-mediated therapy, we blocked this interaction in Th1 cell-treated animals with the anti-CD40L mAb MR-1. This mAb completely abolished the capacity of anti-A20 Th1 cells to cure established lymphomas (Fig. 3C). Although other models of CD40-mediated tumor immunity suggest a crucial role for tumor-specific CD8⁺ T cells (18) and for Abs directed against tumor epitopes (5), these two modes of antitumor immunity seem to be of minor importance in our model. Transfer of A20-

⁵ O. Egeter and M. Röcken, unpublished data.

reactive Th1 cells controlled the tumor in about 3 days, a time too short to provide help for immunoglobulin production or cytotoxic T-cell responses. Consequently, injection of anti-CD8 mAb barely influenced the efficacy of Th1 cell-mediated tumor therapy (data not shown), and significant Ab titers against A20 cells could not be detected (data not shown). Thus, Th1 cells may kill A20 cells through direct CD40-CD40L interactions or indirect, IFN- γ -dependent mechanisms (4, 7). To address the role of Th1 cell-derived IFN- γ in adoptive tumor therapy, we inhibited IFN- γ with the XMG-1.2 mAb *in vivo* according to a protocol that inhibits Th1 cell-mediated protection in murine leishmaniasis (21). The therapeutic efficacy of A20-reactive Th1 cells was completely abrogated (Fig. 3D), strongly suggesting that adoptive therapy with Th1 cells was dependent on both CD40-CD40L interactions and IFN- γ .

Th cells express CD40L (14, 20), and A20-reactive Th1 cells kill A20 lymphomas *in vitro* in a Ca²⁺-independent fashion (Fig. 1D). Because binding of CD40 may lead to direct tumor elimination (18), our findings can be interpreted as direct killing of the A20 lymphoma by CD40-binding Th1 cells. However, the data showing that A20-specific Th0 cells producing both IL-4 and IFN- γ were ineffective in curing mice from established lymphomas and that anti-IFN- γ mAb abolished the therapeutic effect strongly suggest that other mechanisms were critical for tumor elimination by Th1 cells. Signaling through CD40 and IFN- γ activates APCs such as macrophages and primes these cells for initiating inflammatory immune responses (4, 7, 17, 21–23). Because this priming of APCs is inhibited by IL-4 or anti-IFN- γ , the IL-4-producing Th2 or Th0 cells may have inhibited tumor elimination by suppressing the development of proinflammatory macrophages, a mechanism originally described for inflammatory autoimmune and parasitic diseases (8, 21).

CpG ODNs Allow the Rapid Generation of Tumor-specific and Protective Th1 Cells from Mice with Large Tumor Burden. In the experiments described above, A20-reactive Th1 cells were derived from preimmunized animals. To mimic a situation more close to the clinic, we investigated whether anti-A20-reactive Th1 cells could also be generated from mice that had to be euthanized because of tumor wasting (>day 30). In some models, tumors eliminate or silence (10) tumor-reactive T cells, whereas in other transgenic models, tumor-reactive T cells could be readily derived from tumor-bearing animals (24). We first analyzed whether A20-reactive CD4⁺ T cells could be detected in lymphoma-bearing mice. Surprisingly, using CpG ODNs, we could readily generate A20-specific, cytotoxic T cells, even from animals with a large tumor burden (Fig. 4A). Whereas few or no tumor-specific Th1 cells were obtained from cultures initiated in the absence of CpG ODNs (Fig. 4B), the addition of CpG ODN 1668 as an *in vitro* adjuvant allowed us to generate large numbers of A20-reactive Th1 cells even from animals with large tumors. These Th1 cells specifically recognized A20 lymphomas and produced the same cytokine pattern as Th1 cells derived from immunized animals (Fig. 4C). Importantly, these Th1 cells also rejected A20 lymphomas (Fig. 4D), although preliminary results suggest that Th1 cell lines from tumor-bearing animals may be less effective than Th1 cells derived from BiV trioma-immunized animals (compare Figs. 2A and 4D).

Concluding Remarks. The data presented show that CpG ODNs provide a unique *in vitro* adjuvant to rapidly generate highly tumor-specific Th1 cells, even from mice with a large tumor burden. The Th1 cells killed A20 lymphomas in a Ca²⁺-independent fashion, and adoptive transfer of syngeneic, A20-specific Th1 cells was highly efficient in tumor prevention and even in the therapy of advanced A20 lymphomas (day 7). The therapeutic effect was largely independent of CD8⁺ T cells and was strictly dependent on tumor-specific cells with a Th1-phenotype, a high IFN- γ :IL-4 ratio, CD40-CD40L interactions, and IFN- γ . These data suggest that Th1 cells eradicated the estab-

lished tumors predominantly through an indirect mechanism. Because Th1 cells control intracellular pathogens through the activation of macrophages (21), and because activation of macrophages by IFN- γ was recently shown to be important in tumor prevention (7), it is likely that Th1 cells use this CD40L- and IFN- γ -dependent mode of cell-mediated immunity for the elimination of established tumors.

Surprisingly, these mechanisms of tumor elimination differ substantially from those recently reported by Nishimura *et al.* (25), who found that both Th1 and Th2 cells are equally effective in tumor therapy. Moreover, in the system reported by Nishimura *et al.*, Th1 cells strictly required tumor-specific CD8⁺ T cells to eradicate the tumor and killed lymphoma cells exclusively in a Ca²⁺-dependent fashion. Importantly, these two systems differ profoundly in their approach. Nishimura *et al.* used T-cell receptor-transgenic Th cells directed against OVA-transfected tumor cells. In contrast, we used no artificial rejection antigen and derived tumor-specific Th1 cells from nontransgenic, tumor-bearing animals by stimulating CD4⁺ T cells in the presence of CpG ODNs. Establishing whether these fundamentally different modes of tumor immunity are determined by the source of the Th, by the immunostimulatory ODNs, or by the routes of tumor application (s.c. versus i.p. or i.v.) may provide important data for the development of tumor therapies.

Importantly, tumor elimination was never associated with autoaggressive disease, showing that tolerance to self-proteins, whose expression pattern is shared between A20 cells and normal tissue, was not broken, even when A20-specific Th1 cells were derived from trioma-immunized animals. Because tumor-reactive Th1 cells can be generated not only from immunized animals but also from tumor-bearing animals, adoptive transfer of tumor-specific Th1 cells may provide a novel and safe therapeutic approach for hematopoietic tumors and perhaps even solid tumors.

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