Immunization with Wild-Type p53 Gene Sequences Coadministered with Flt3 Ligand Induces an Antigen-specific Type 1 T-Cell Response

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ABSTRACT

We examined the ability of immunization with sequential adenovirus/plasmid DNA vectors expressing human wild-type p53 to stimulate a type 1 T-cell response and induce protection against challenge from a metastatic tumor that expresses mutated murine p53. We found that tumor protection and an antigen (Ag)-specific immune response were enhanced by prior injection of Flt3 ligand (Flt3L) at a dose and schedule that significantly increased dendritic cell (DC) number and frequency. Preliminary studies using enzyme-linked immunospot and Winn assays suggested that Ag-specific CD8+ cells, with their significant increase in IFN-γ-secreting activity (Tc1 cells), were responsible for the tumor protection. The delayed-type hypersensitivity response to p53 was increased in mice immunized with p53 alone or p53 and Flt3L compared with a negative control. In contrast, spleen cells from mice immunized with p53 and Flt3L exhibited a higher Ag-specific proliferative response than mice immunized with p53 alone. The frequencies of Ag-specific IFN-γ- and interleukin (IL)-4-secreting cells were determined using an enzyme-linked immunospot assay, which demonstrated that the frequency of IFN-γ-secreting cells was significantly higher in mice immunized with p53 and Flt3L than in mice receiving Flt3L, excipient, or p53 treatment alone. In contrast, the frequency of IL-4-secreting cells did not differ significantly among these groups. We also observed an increased frequency of IL-12 and IFN-γ-secreting cells (but not IL-4 or IL-10) in the spleens of mice immediately after 10 days of Flt3L treatment, which was also the day of p53 priming. This observation supports the likelihood that there are multiple mechanisms of Flt3L adjuvant activity, including expansion of DC and type 1 T-cell number. Overall, these results suggest that immunization with p53 genetic sequences after in vivo expansion of DC, using Flt3L, provides a useful strategy to induce p53-specific, and protective, type 1 T-cell responses.

INTRODUCTION

The induction of Ag2-specific, cell-mediated immune responses to tumors is a promising approach for adjuvant cancer therapy. The tumor suppressor gene, p53, is mutated and subsequently overexpressed in >50% of human malignancies, making it an attractive target for tumor immunotherapy. Lung, breast, and colon cancers are among the solid tumors that frequently have p53 mutations (1, 2). However, p53 mutations occur at various sites within the molecule, requiring the identification of each patient’s p53 mutation before therapy. Furthermore, not all mutations are found within the MIC-binding epitopes, and a patient’s primary tumor and metastasis can express different p53 mutations (3), further limiting mutation-specific p53-based immunotherapy. However, the mutations result in increased levels of p53 protein processing and presentation of wild-type p53 epitopes not normally seen with nonmutated protein (4, 5). Thus, wt p53 sequences can potentially be used in immunotherapy by making the entire p53 sequence available as an Ag. This results in wt p53 peptide-specific CTLs and antibodies that can recognize p53-overexpressing tumors (4–7).

The cellular arm of the immune system is controlled in part by type 1 T cells, which secrete IL-2 and IFN-γ and represent the helper and effector arm of the T-cell immune response. Conversely, type 2 T cells mediate humoral immunity and express a cytokine profile, including IL-4 and IL-10, that can inhibit T-cell response. Professional Ag-presenting cells, or DCs, also play a key role in regulating the type and extent of immune response (8, 9). One mechanism of immune impairment in tumor-bearing hosts has been linked to a defect in Ag processing and presentation (10, 11). Thus, one approach toward successful immunotherapy is to identify an adjuvant to enhance Ag presentation and selectively induce a type 1 immune response. Recent interest has focused on DCs as cellular adjuvants in tumor immunotherapy because of their capacity to process and present Ag to T cells (11–17). It has been shown that DCs can be significantly expanded in vivo by repeated injections of Flt3L (18–20). It has also been suggested that the injection of Flt3L results in an Ag-specific, type 1 cell cytokine response in mice (21). However, it is unclear whether expansion of DCs in vivo could increase an antitumor immune response.

Viral vectors have been used to efficiently deliver wt p53 in vivo (22, 23). However, the presence of neutralizing antibodies precludes boosting using the same viral vector (11, 15, 24, 25). Furthermore, there is an associated potential for nonspecific stimulation of the immune system with viral vectors (11, 24, 26). Immunization with naked DNA (nonviral) is also used to induce a specific immune response against tumor Ags. However, compared with viral vectors, plasmid vectors induce lower Ag levels and result in a decreased T-cell response. Conversely, naked DNA may generate a smaller inflammatory response than live viral vectors (11, 16, 26). Therefore, to overcome these drawbacks, a heterologous prime-boost strategy with Adv-p53 and plasmid-p53 constructs was developed.

We report herein that a priming immunization with Adv-p53 and boosting with a p53 plasmid DNA induced a p53-specific, type 1 T-cell response in vivo, greater than that associated with either vector alone. Furthermore, expansion of DCs using the injection of Flt3L before immunization with p53 gene sequences enhanced the tumor-specific type 1 response resulting in protective immunity to challenge with a metastatic mammary adenocarcinoma with high p53 (murine) expression. This strategy should be useful for the treatment of tumors with mutated p53 regardless of the mutation.

MATERIALS AND METHODS

Mice and Immunization Protocol. Female BALB/cJ (H2b) mice, 6–8 weeks of age, were purchased from Charles River (Wilmington, MA) and allowed to acclimate for 2 weeks before use. Mice were immunized i.m. with wt human Adv-p53 (107 viral particles/mouse) on day 0 and boosted with...
human p53 plasmid (100 μg/mouse, i.m.) on days 14 and 28. The human p53 plasmid, with a mutation at codon 175 resulting in an arginine to histidine amino acid substitution, under the control of the cytomegalovirus immediate early gene promoter (27), was provided by Dr. B. Vogelstein of Johns Hopkins Oncology Center, Baltimore, MD. The mutated p53 sequence was used to stabilize the protein and increase the immunogenicity of the protein as an Ag. The Adv-p53 vector, expressing wt human p53 and driven by the cytomegalovirus immediate early gene promoter (28), was kindly provided by Canji, Inc., San Diego, CA. Recombinant human Flt3L, a kind gift from Immunex (Seattle, WA), was administered daily (i.m.) for 10 days at 10 μg/animal (A) 10 days before immunization (day 10 to day 1) and again before the first boost (day 4 to day 13). Our preliminary investigations revealed that this protocol of Flt3L administration was optimal for DC expansion before immunization.

Preparation of Tumor Cells. 4T1 cells, a murine metastatic breast cancer cell line that is p53 null (29), were provided by Dr. Fred Miller, Michigan Cancer Foundation, Detroit, MI, and transfected with human p53 plasmid DNA using LipofectACE reagent (Life Technologies, Inc., Gaithersburg, MD) following the manufacturer’s instructions. Transfected cells were selected in 500 μg/ml G418-sulfate (Geneticin, Life Technologies, Inc., Gaithersburg, MD) containing media and were subsequently continuously maintained in 500 μg/ml G418-sulfate-containing media. The expression of p53 in 4T1/p53 transfected (clone 1) was confirmed by a Western blot analysis using Ab-7 (Oncogene, Cambridge, MA; Fig. 1). The p53-transfected and nontransfected 4T1 cells were therefore referred to as 4T1/p53 and 4T1 cells, respectively. The challenge study used c166, which expresses high levels of murine p53 (30, 31). 4T1 and c166 are separate cell lines from the same parent tumor (32).

Synthesis of p53 Peptides. p53 nonamers, based on Parker’s algorithms (33) which fit into the Class I groove of H2b mice, P15 (MFRELN)EAL and P16 (TFSDLWKLL), were synthesized and purified by Quality Controlled Biochemicals, Inc. (Hopkinton, MA) using 9-fluorenlymethoxycarbonyl solid phase chemistry and purified using a Gilsen automated high-performance liquid chromatography system.

DTH Response. Two weeks after the last immunization, 4 μg of p53 peptides (2 μg each of P15 and P16) in a volume of 30 μl were injected into the pinna. Twenty-four h after challenge, the edema of the test and control (PBS) contralateral ears were measured using a Mitutoyo (South Plainfield, NJ) No. 7300-gauge caliper. To determine the edema level, the thickness of the saline-injected control ear was subtracted from the test ear thickness.

IVS of Splenocytes. Splenocytes (3–4 × 10^7 cells/10 ml) from each immunized and control mouse were separately stimulated for 5 days in six-well plates with 3–4 × 10^4 irradiated normal splenocytes, preincubated for 2 h with p53 nonamer peptides (10 μg/ml) in complete media composed of Click’s media (Life Technologies, Inc.) supplemented with 0.5% normal mouse serum, 20 μg/ml gentamicin, 4 mM L-glutamine, and 5% rat T-Stim without Con A (Collaborative Biomedical Products, Bedford, MA). After a 5-day IVS, the cells were transferred into 96-well plates, with or without Ag, for lymphocyte proliferation assays.

**RESULTS**

DTH Response in Mice Immunized with p53 and Flt3L. In these studies, the mice were primed with 10^9 Adv-p53 particles and boosted
on days 14 and 28 with 100 μg of p53 plasmid DNA injected i.m. The Flt3L cohorts received 10 daily injections of Flt3L (10 μg) before immunization. Cohorts of mice immunized with p53 alone and p53 and Flt3L showed a significantly higher DTH response relative to the Flt3L alone or HBSS-treated cohorts (Fig. 2). However, there was no difference in the DTH induced by p53 treatment alone as compared with p53 and Flt3L administration.

**In Vitro Proliferation of Lymphocytes in Response to p53 Ag with or without IVS.** Splenocytes from animals immunized with a combination of Adv-p53 and plasmid p53, with or without Flt3L, were tested for their lymphoproliferative response before or after an IVS with p53 nonapeptides. The splenocytes from mice injected with p53 gene sequences showed significantly higher lymphoproliferation SI [SI = 3.4 ± 0.1] compared with the lymphocytes from mice injected with HBSS (SI = 1.0 ± 0.1; Fig. 3). The proliferative response was augmented further in groups treated with both p53 and Flt3L (SI = 6.7 ± 2.4) relative to the control groups and the p53-alone group or Flt3L-alone group (SI = 3.5 ± 1.1). In other experiments, the splenocytes were stimulated *in vitro* for 5 days in the presence of p53 peptides before being assayed for lymphoproliferation.

[Fig. 2. DTH response to p53 peptide after immunization with p53 and Flt3L. Mice were immunized with p53 genetic sequences and Flt3L. Two weeks after the final immunization, mice were challenged with 4 μg of p53 nonapeptides (2 μg each of P15 and P16) into the right pinna. After 24 h, the thickness of test and control ears were measured. Results are shown as the difference in ear thickness between the test and control ears. Data are representative of five independent experiments with four mice each. *P < 0.05 versus cohorts immunized with HBSS alone. †, P < 0.05 versus cohorts immunized with Flt3L alone.](image)

[Fig. 3. p53 Ag-specific lymphocyte proliferation before or after IVS with p53 peptides. Mice were immunized with p53 gene sequences and Flt3L. Two weeks after the final immunization with p53, mice were killed and splenocytes harvested. A, one set of splenocytes was directly plated (time 0) into 96-well microtiter plates with 10% irradiated 4T1 or 4T1/p53 cells and incubated for 5 days. †, another set of splenocytes was stimulated *in vitro* (IVS) for 5 days in the presence of 10% irradiated normal splenocytes and 20 μg/ml p53 peptides. After the IVS, the splenocytes were incubated for 5 days in 96-well microtiter plates with 10% irradiated 4T1 or 4T1/p53 cells as stimulators. In both cases, [3H]thymidine was added at 1 μCi/well 16–18 h before cell harvest. Cultures were harvested onto fiberglass plates and [3H]thymidine incorporation was quantified by scintillation counting. The p53 Ag-specific proliferation is expressed as the mean SI calculated as described in “Materials and Methods.” Data are representative of three independent experiments, four mice each, done in triplicate. *P < 0.05 versus cohorts immunized with HBSS alone. †, P < 0.05 versus cohorts immunized with Flt3L alone. ‡, P < 0.05 versus cohorts immunized with p53 alone.](image)

[Fig. 4. Comparison of combination versus single-agent immunization with Adv- and/or plasmid-p53 in a p53 Ag-specific lymphocyte proliferation assay. Mice were injected with Flt3L and then immunized with Adv-p53 and/or p53 plasmid. Each group of animals received the same number of immunizations. Two weeks after the final immunization with p53, mice were killed, and splenocytes were harvested and directly plated into 96-well microtiter plates with 10% irradiated 4T1 or 4T1/p53 cells and incubated for 5 days. [3H]thymidine was added at 1 μCi/well 16–18 h before cell harvest. The p53 Ag-specific proliferation was determined as above. Data are representative of two independent experiments, four mice each, done in triplicate. *P < 0.05 versus cohorts immunized with HBSS alone. †, P < 0.05 versus cohorts immunized with Flt3L alone. ‡, P ≤ 0.05 versus cohorts immunized with a single p53 vector type.](image)
we performed an ELISPOT assay for IFN-γ- and IL-4-secreting cells. As shown in Fig. 5, there was a significantly higher frequency of IFN-γ-secreting cells in the splenocytes of mice immunized with p53 and Flt3L compared with all other groups, including mice immunized with p53 alone. Moreover, the frequency of IFN-γ-secreting cells was enhanced only in groups stimulated with 4T1/p53 cells, as opposed to stimulation with 4T1 cells. This provides a control indicating that this response was p53 Ag-specific. In contrast, there was no significant difference in the frequency of IL-4-secreting cells among p53- and Flt3L-treated or p53-immunized groups of mice (Fig. 5). This result confirmed that the T-cell response observed after p53 and Flt3L immunization was a type 1 response and p53 Ag-specific.

**Effect of Flt3L Administration on the Frequency of IL-12-, IL-10-, IFN-γ-, and IL-4-secreting Cells in Splenocytes.** To examine the mechanism of Flt3L adjuvant activity, the frequency of IL-12-, IL-10-, IFN-γ-, and IL-4-secreting cells was determined by ELISPOT assay 24 h after 10 daily injections of Flt3L (10 μg/day, i.m.). The splenocytes were harvested on day 11 and ELISPOT assays performed in the presence of irradiated splenocytes (feeder cells) without stimulation by any mitogen or specific Ag. As shown in Fig. 6, the frequencies of IL-12- and IFN-γ-secreting cells were significantly higher in mice treated with Flt3L (10-fold and 8-fold increase, respectively) compared with those in HBSS-treated mice. In contrast, the frequencies of IL-10- or IL-4-secreting cells were not changed by administration of Flt3L (Fig. 6).

**Survival of Mice Challenged with Murine p53-expressing Tumor, cl66, after Immunization with p53 and Flt3L.** As shown in the results above, a type 1 immune response was generated in mice after immunization with p53 and Flt3L, as determined using human p53-transfected cells. However, whether this type 1 immune response against p53 would translate into effective antitumor response in vivo, especially against murine p53, is not addressed. Therefore, we performed a study where mice were immunized with p53 and Flt3L and challenged 1 week after final immunization by s.c. injections of the murine mammary tumor cells, cl66, which have a high expression of mutated p53 (29, 30). As shown in Fig. 7, mice treated with Flt3L or p53 alone had prolonged survival compared with the control group. However, survival was significantly enhanced by the coadministration of p53 and Flt3L, with complete rejection of tumor in 40% of mice in the p53- and Flt3L-injected groups (Fig. 7). The surviving mice, as well as control mice, were again challenged with cl66 (10^6 cells, s.c.) on day 120. In the secondary challenge study, the control mice developed tumors, whereas the immunized mice had none (data not shown). When these mice were killed, the splenocytes of the mice that were p53-immunized and challenged with cl66 had a significantly higher CTL response against 4T1/p53 cells compared with the control mice (data not shown). This study also confirms the p53-specificity of the immune response. One interesting part of this study is that the mice were challenged with a murine p53 overexpressing tumor, al-

![Fig. 5. Frequency of IL-12- and IL-4-secreting cells in the splenocytes after immunization with p53 gene sequences and Flt3L. Mice were immunized with p53 genetic vaccines and Flt3L. Two weeks after the final immunization with p53, mice were killed, and splenocytes were stimulated in the presence of 10% irradiated 4T1 or 4T1/p53 cells. After 48 h, the frequency of IFN-γ- and IL-4-secreting cells were determined by ELISpot assay. Data are mean ± SE of three independent experiments, four mice each, done in triplicate. *P < 0.05 versus cohorts immunized with Flt3L alone. †P < 0.05 versus cohorts immunized with Flt3L alone.](image1)

![Fig. 6. Frequency of type 1 and type 2 cytokine-secreting cells in the splenocytes immediately after treatment with Flt3L. Flt3L was administered (10 μg/animal/day) for 10 days. On day 11, mice were killed and splenocytes were harvested, and ELISPOT assay was performed to determine the frequency of IL-12-, IL-10-, IFN-γ-, and IL-4-secreting cells. Data are representative of two independent experiments, four mice each, done in triplicate. *P < 0.05 versus cohorts injected with HBSS alone.](image2)
though immunization was against human p53, demonstrating an immune cross-reaction between human and murine p53.

**Effector Cells Induced after Vaccination with p53 and Flt3L**

Using ELISPOT and Winn Assays. Studies were initiated to examine the effector cell(s) responsible for protection to tumor challenge and the cytokine-producing cells in the ELISPOT assay. A vaccination protocol similar to that used in the tumor-challenge study (Fig. 7) was undertaken (prime with Adv-p53 and two p53 plasmid boosts with Flt3L administration before the prime and first boost). Three weeks after the last boost, CD3⁺CD4⁺ cells and CD3⁺CD4⁻ cells (CD8⁻) were fractionated using the Miltenyi MiniMacs system and the cell function examined by ELISPOT (without IVS) and Winn assays (36). The CD8⁻ cells were isolated by negative selection to prevent inclusion of CD8⁻ NK cells, and purity was determined by flow cytometric analysis, which was found to be ≥95%. The unfractionated spleen cells from the vaccine alone and the vaccine and Flt3L cohorts had a significant increase in the frequency of IFN-γ-secreting cells compared with both the control (HBSS) and Flt3L-treated cohorts (Fig. 8). The CD4⁺ cells obtained from the vaccine and Flt3L cohort also had a significant increase in IFN-γ-secreting cells, although CD4⁺ cells from the vaccine alone-treated mice did not. In contrast to the unfractionated cells, IFN-γ-secreting CD4⁺ cells from the Flt3L-treated mice were significantly increased, suggesting a nonspecific increase caused by Flt3L administration. Isolated CD8⁺ cells had a significant increase in IFN-γ-secreting cells in both vaccine-treated groups (with or without Flt3L) compared with the control (HBSS) and Flt3L cohorts. In a concomitant Winn assay, results shown as a Kaplan-Meyer plot (Fig. 9), unfractionated spleen cells admixed with c66 cells had no impact on the survival of the injected mice, resulting in median survival times ranging from 49 to 54 days. Similarly, coadministration of c66 cells with CD4⁺ cells from mice injected with Flt3L before p53 vaccination did not significantly prolong survival compared with control animals (median survival time, 57 days). In contrast, the admixture of c66 cells with CD8⁺ cells from mice immunized with p53 and Flt3L cured 34% of mice, with a significant prolongation of survival resulting in a median survival time of 71 days compared with the HBSS control (59 days). There was also a significant increase in survival relative to the mice treated with Flt3L alone, which had a median survival time of 53 days. Both the CD4⁺ and CD8⁺ cells from the Flt3L-alone-treated mice had a trend toward a decreased median survival time relative to the mice injected with spleen cells from HBSS-treated mice. This achieved significance with the CD4⁺ cells from Flt3L-treated mice, which, after injection, resulted in a median survival time of 27 days as compared with 57 days for mice receiving CD4⁺ cells from HBSS-injected mice.

**DISCUSSION**

The strategies of in vivo expansion of DC using Flt3L and immunization with wt human p53 using a sequence of Adv and plasmid DNA were examined in this study. We demonstrated the induction of an Ag-specific type 1 T-cell immune response in vitro as well as protection to tumor challenge with a murine tumor overexpressing p53. We observed a high DTH response to p53 peptides in mice immunized with sequential p53 genetic sequences (Adv-p53 and p53 plasmid DNA) and Flt3L; however, the DTH response did not differ from the group immunized with p53 genetic sequences alone. This suggests that the maximum DTH response might have been induced by p53 genetic priming alone and could not be augmented further by Flt3L. In contrast, we observed significant T cell proliferation in p53-immunized cohorts in response to 4T1/p53 cells. This T-cell
observed cytokine profile indicated that a type 1 response was induced with a high frequency of IFN-γ-secreting cells in groups injected with Flt3L and then immunized with p53 genetic sequences. No cytokine response was observed in groups stimulated with 4T1 cells, once again confirming that the response was p53-specific. The ELISPOT responses observed were also in agreement with the results of an Ag-specific T-lymphocyte proliferation assay.

DCs, expanded in vitro from bone marrow cells and pulsed with wt or mutant p53 peptides, have been used to induce protective or therapeutic immune response against p53-expressing tumors (37). Moreover, immunization of mice with genetically modified DCs, after bio-ballistic introduction into DCs with a plasmid DNA encoding the murine p53 minigene, was found to be as effective as peptide-pulsed DC in protection from subsequent tumor challenge (reviewed in Ref. 44). Our results, however, suggested that DC, expanded in vivo by Flt3L, provided in situ adjuvant activity to p53 Ag and primed T cells in vivo. The exact mechanism of Ag uptake and processing by DC after administration of Adv-p53 and p53 plasmid DNA and the presentation to T cells remains unclear. There are reports that DC can capture either protein secreted by other cell types that were transfected after genetic immunization or Ag from transfected cells in a poorly defined mechanism referred to as “cross-presentation” (38). Additionally, a DC population may be transfected itself (39–41). The transfected or Ag-expressing DC then migrate to nearby lymph nodes, where they present the Ag to naïve T cells.

Flt3L injected into mice, before or after the injection of tumor, has been reported to inhibit tumor growth in vivo (19, 20, 42–44). In addition, there are reports that transfer of splenic T cells from tumor-free animals after immunization with either GM-CSF or Flt3L-expressing tumor cells protected naïve animals from subsequent parental tumor challenge (20, 43). Moreover, immunization with Flt3L-transduced tumor cells seemed to be more potent in inhibiting tumor growth upon subsequent challenge compared with immunization with GM-CSF-transduced tumors. The authors attributed this higher potency of Flt3L to enhanced stimulation of NK activity by Flt3L compared with that by GM-CSF (43). The mechanism of T-cell augmentation by Flt3L, however, has not been established. It has been reported that Flt3L administration enhances the CD11c-highCD11b-low type DC, which leads to an Ag-specific, type 1 T-cell response (21). We observed that immediately after Flt3L administration (10 daily injections) there was a 14-fold increase in splenic DCs, with a 16-fold increase in cells expressing CD11c-highCD11b-low (type 1) phenotype, which was significantly greater than the 11-fold increase in the CD11c-highCD11b-high (type 2) phenotype. There was also a significant increase in the frequency of IL-12-secreting cells in the spleens of mice treated with Flt3L compared with those treated with HBSS alone. This was accompanied by a higher frequency of IFN-γ-producing type 1 cells in the spleens. However, the frequency of type 2 (IL-10 or IL-4) cytokine-secreting cells remained unaltered by Flt3L administration. This alteration of type 1 cytokine response was observed immediately after Flt3L administration and gradually disappeared over a period of 2 weeks (data not shown). Therefore, the significant augmentation in an Ag-specific, type 1 T-cell response in the present report was likely, at least in part, because of the induction of a “type 1 bias” by administration of Flt3L before p53 priming. Codelivery of type 1-biasing cytokines, IL-12 and IFN-γ, have also been demonstrated to enhance the efficacy of immunization with p53 peptides (45, 46) or peptide-pulsed DC (47).

During these studies, we detected a low CTL response (around 15% at an effectortarget ratio of 25:1) against p53–4T1 cells or P815 cells pulsed with p53 peptides, after IVS of immunized splenocytes for 5 days (data not shown). However, we were not confident that the immunity induced was at a biologically relevant level. Furthermore,
the Ag used was derived from the human p53 gene sequence, and although the sequences are conserved, we questioned whether protection to murine p53 expression could be obtained. We, therefore, investigated whether the induction of a type 1 cytokine response and lymphoproliferation could translate into protection from tumor challenge. In these studies, mice immunized with human p53 genetic sequences, with or without Flt3L, were challenged with murine breast carcinoma cells, c66, expressing mutated p53. The cohort of mice that received p53 and Flt3L survived significantly longer, with 40% tumor-free survival, compared with HBSS, Flt3L, or p53 alone controls. Furthermore, the surviving mice completely rejected tumor cells upon rechallenge with c66, injected on day 120 (data not shown), which suggests that Ag-specific memory was induced. Thus, the poor CTLs observed in vitro may be attributable to an IVS with only p53 nonapeptides designed to fit into the groove of MHC class I molecules, and thus inadequate class II Ag expression. This would result in inadequate CD4+ T cell help, which is necessary for the stimulation of CD8+ CTLs (48). In other studies of tumor challenge, we observed significant survival prolongation, but a lower cure rate. This suggests that additional optimization of the protocol is needed before entering into therapeutic studies.

Preliminary studies into the effector cells responsible for the tumor-protective activity and the responding cells suggested that Ag-specific Tc1 cells were the primary responders in the ELISPOT assay. In addition, Tc1 cells seemed to be responsible for the tumor-protective activity as isolated CD8 cells, with their significant increase in Ag-specific IFN-γ-secreting activity as isolated CD8+ cells in the absence of T cells, with their significant increase in Ag-specific IFN-γ-secreting activity as isolated CD8+ cells in the presence of T cells, as demonstrated in a study with mice immunized with p53 in the presence of Flt3L, as compared with the significant increase in “cured” mice after the injection of isolated CD8+ cells from the cohort immunized with p53 with Flt3L, may be attributable to an enrichment in the number of CD8+ cells capable of responding to the tumor. There was also the suggestion of an increased frequency of Ag-specific IL-4-secreting cells in the isolated CD4+ T cell population in response to Flt3L (data not shown), which may contribute to the lack of protection with unfractonated cells. Although, there was also an increase in Tc2 cells, the frequency was much lower than that observed with Th2 cells, potentially allowing Tc1 effector activity. The Wen essay confirms the participation of Tc1 cells in the antitumor response in our study. However, the potential role of additional effector cell types, like NK cells, is not ruled out.

In summary, we report the stimulation of a p53-specific, type 1 immune response in mice after immunization with p53 and Flt3L. The immunization protocol was also effective in preventing the growth of murine tumors and prolonging the survival of hosts upon challenge with a p53-expressing tumor. These results demonstrate that immunization with p53 following in vivo expansion of DCs with Flt3L can be an effective strategy to generate p53-specific, type 1 T-cell responses to tumors. Current studies include a focus on a comparison of the efficacy of different injection routes with Adv-p53 (i.v. versus i.m.). We are also comparing different plasmid vectors for Flt3L DNA and the feasibility of one or two injections of Flt3L-plasmid DNA as an alternative to 10 daily injections of Flt3L for the expansion of DCs.

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