Evaluation of cellular immune responses in rhesus monkeys subjected to adenovirus-mediated gene transfer into the cervix

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We reported previously that direct injection of a recombinant adenovirus (rAd), Ad5CMV-β-gal, into the cervix of the rhesus monkey resulted in efficient β-galactosidase expression in the cervix within 3 days. In these studies, we also observed the induction of anti-adenovirus (Ad)-specific immunoglobulin G responses after 22 days. In the continuation of evaluating the anti-Ad-specific immune responses resulting from this approach of gene targeting to the cervix, we measured the cellular immune responses. The introduction of Ad5CMV-β-gal into the cervix by direct injection, but not by the abrasion technique, resulted in the induction of strong proliferative responses against extracts of cells infected with Ad5CMV-β-gal but not against control uninfected cells. These responses were initially detected at 22 days postinjection and coincided with the abrogation of transgene expression. Significant levels of proliferative responses were maintained for ≤83 days. Multiple injections of rAds had no significant enhancing effect on either the level or longevity of the proliferative responses. At 3 days after the injection of Ad5CMV-β-gal, when the transgene expression in the cervix was clearly evident, proliferative responses against the rAd were not detectable. However, the production of low but significant amounts of interleukin-10, a cytokine characteristic of T helper type 2 responses that promote humoral immune responses, was observed at the 3-day point in these animals. These results suggest that significant differences exist between the kinetics of transgene expression and the priming of specific host immune responses, and that these differences may be important for devising alternate strategies to improve techniques for Ad-mediated gene therapy of cervical cancer.

Key words: Cervical cancer; recombinant adenovirus; gene therapy; cellular immunity.

Cervical cancer represents the second most common malignancy affecting women worldwide.[1] Epidemiological studies suggest that infection by human papilloma virus (HPV) is a major risk factor for the development of cervical neoplasia.[2] The presence of HPV types 16 and 18 has been observed in ≤90% of cervical cancers.[3,4] In certain HPV-associated cervical cancers, the inactivation of p53 and Rb, two major tumor-suppressor gene products, occurs through their interactions with the E6 and E7 oncoproteins of HPV, respectively.[5,6] In addition, mutations within the p53 gene represent other genetic aberrations associated with certain forms of cervical cancer.[7] In either case, cervical cancer represents an ideal model for genetic intervention.

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Recombinant adenoviruses (rAds) have emerged as promising vehicles for *in vivo* gene transfer to a wide variety of cells.[8–10] Previous *in vitro* and *in vivo* studies by our group showed that the introduction of Ad5CMV-p53, a rAd encoding a normal p53 gene, results in suppression of the growth of human cervical cancer cell lines.[11] Encouraged by these observations, we have recently conducted a preclinical feasibility study in rhesus monkeys for direct introduction of rAd in the cervix and to monitor the expression of the transgene (β-galactosidase (β-gal) and/or p53). In these studies, we observed efficient transgene expression when the rAd was introduced into the cervix by direct injection as opposed to by the abrasion technique.[12]

Because sustained expression of the transgene in the cervix may be necessary for a favorable therapeutic outcome of the cervical cancer, repetitive introduction of the rAd may be necessary. In this regard, the major problem associated with adenovirus (Ad)-mediated gene delivery in an immunocompetent host is the potential for the abrogation of transgene expression due to the induction of a strong host immune response directed against

Table 1. Adenovirus-Specific Proliferative Responses in Rhesus Monkeys Subjected to Abrasion for Introducing Ad5CMV-β-gal into the Cervix

Monkey no.	Dose (PFU)	Proliferative response (SI)		
		day 0	day 3	day 49
J9	2×10^8	1.0	1.1	1.1
L163	2×10^9	0.9	0.9	1.2
L879	2×10^9	1.0	1.0	0.9
J47	2×10^{10}	1.1	1.3	0.8
L745	2×10^{10}	1.1	1.1	1.0

the Ad vectors and possibly also due to the gene of interest. Several groups that attempted rAd-mediated gene delivery to different organs reported that expression of the transgene diminished as a result of memory-type immune responses in the host.[13–15] As reported previously,[12] we observed an efficient induction of a humoral immune response against the adenoviral vector in rhesus monkeys following the injection of rAds carrying β-gal and/or p53 into the cervix. In continuation of our studies in the rhesus monkey model for cervix-directed gene transfer, we have attempted to understand the immunological consequences by determining the development of Ad-specific, cell-mediated immune responses and the longevity of such immunity.

MATERIALS AND METHODS

Recombinant Ad

The rAds used in this study, Ad5CMV-β-gal and Ad5CMV-p53, have been described previously.[12] Briefly, the Ad vector contains the cytomegalovirus promoter, simian virus 40 polyadenylation signal, and either β-gal or p53 in a minicassette inserted into the E1-deleted region of modified Ad5.[11] The rAd stocks were prepared in 293 cells. Cells were harvested at 36–40 hours postinfection and lysed; next, the virus was purified by ultracentrifugation on a double CsCl gradient as described previously.[11] The virus titer was determined by a standard plaque assay.

Animal experiments

The physical properties and the clinical management details of the rhesus monkeys used in the study were reported previously.[12] Briefly, a total of 15 monkeys (three groups of five animals each) were used for this study. The monkeys were kept individually in single cages in our animal care facility according to "Animal Care and Biosafety Guidelines" of the University of Texas M.D. Anderson Cancer Center. Five monkeys in group I (J9, L163, L879, J47, and L745) received three different doses of the rAd, Ad5CMV-β-gal, by the abrasion technique (Table 1). Monkeys in group II (L571, L741, L87, L345, and L577) received the same three different doses of Ad5CMV-β-gal by direct injection into the cervix (Table 2). The monkeys in group III (L515, J23, L559, L567, and J3) were injected with a mixture of Ad5CMV-β-gal and Ad5CMV-p53 (each at 1×10^{10} plaque-forming units (PFU)) on days 0, 28, and 56 (Table 3). Heparinized blood samples were collected from the monkeys at regular intervals following insertion of the adenovector.

T-cell proliferation assay

Peripheral blood mononuclear cells (PBMCs) were separated from the heparinized blood samples of the monkeys by the method described previously.[16] Briefly, freshly obtained blood samples were subjected to Ficoll-Hypaque (Histopaque-1083, Sigma Immunochemicals, St. Louis, Mo) density gradient centrifugation; the PBMC fraction was washed twice with complete RPMI 1640 containing 10% fetal calf serum and resuspended in the same medium at 1×10^6 cell/mL. Aliquots of 0.1 mL in triplicate were incubated with various control and test antigens (Ags). The adenoviral Ag used in this study was

Table 2. Adenovirus-Specific Proliferative Responses in Rhesus Monkeys Injected with Ad5CMV-β-gal into the Cervix

Monkey no.	Dose (PFU)	Proliferative response (SI)				
		day 0	day 3	day 22	day 49	day 83
L571	2×10^8	1.2	0.9	18.9	6.1	3.1
L741	2×10^9	1.5	1.2	ND	9.2	3.6
L87	2×10^9	1.3	1.2	45	2.1	2.0
L345	2×10^{10}	0.8	1.5	41	8.0	4.1
L577	2×10^{10}	1.6	1.2	ND	12.1	5.1

ND, not determined.

Table 3. Adenovirus-Specific Proliferative Responses in Rhesus Monkeys Injected with Ad5CMV-β-gal and Ad5CMV-p53 into the Cervix

Monkey no.	Dose (PFU)	Proliferative response (SI)					
		day 0	day 3	day 31	day 56	day 59	day 155
L515	2×10^{10}	0.9	1.1	5.7	10.1	13.5	1.5
J23	2×10^{10}	1.4	1.0	23.6	18.2	9.8	1.2
L559	2×10^{10}	1.2	1.0	26.1	38.2	5.4	1.1
L567	2×10^{10}	1.3	1.0	15.9	8.2	3.6	1.2
J3	2×10^{10}	1.1	0.7	15.7	11.6	3.0	1.3

the purified virus (Ad5CMV-β-gal) prepared in 293 cells. Cells were harvested at 36–40 hours postinfection and lysed; the virus was purified by ultracentrifugation on a double CsCl gradient as described previously.[11] Before use, the virus was inactivated by incubation at 70°C for 30 minutes. A 20-μL aliquot (1 μg of viral protein) from the 1/100 dilution of the heat-inactivated virus was used in a total volume of 200 μL in triplicate wells of the 96-well, U-bottom microtiter plate. In limited experiments, extracts of 293 cells (6×10^7) that were either uninfected or infected for 2 days with Ad5CMV-β-gal were also used as Ags. Additional treatments included medium and phytohemagglutinin (PHA). Cells were incubated at 37°C for 5 days in a humidified 5% CO_2 incubator, and 1 μCi [^{3}H]thymidine (ICN Biochemical, Costa Mesa, Calif) was added to each well during the final 16–18 hours. Cells were harvested onto filter strips, and radioactive thymidine incorporation was estimated. The significance of the T-cell proliferation response to the Ad was calculated as the fold increase of thymidine incorporation over that of the medium control. A stimulation index (SI) value of ≥2.0 indicated a positive proliferative response, whereas SI values of ≥3.0 were considered significant.

Cytokine production

After determining the Ad-specific proliferative responses, we assayed for the production of various cytokines using the cryopreserved PBMC samples from the monkeys. Due to the availability of only a limited number of samples, we determined the cytokine production in the culture supernatants of PBMCs of three monkeys each in groups II (L571, L741, and L345) and III (L515, J23, and L559) that were injected with Ad5CMV-β-gal alone or in combination with Ad5CMV-p53. PBMCs (1×10^5) from various monkeys were cultured in a total volume of 200 μL of RPMI 1640 with 10% fetal calf serum in a 96-well plate in the presence of either medium, CsCl-purified Ad, 293 cell extract, or extracts of 293 cells harboring virus. The culture supernatants were harvested after 2 days (for interleukin-2 (IL-2) and IL-4 measurements) or 3 days (for IL-10 and interferon-γ (IFN-γ) measurements) as described previously.[17] All cytokine assays were performed using the Cytoscreen enzyme-linked immunosorbent assay kits (Biosource International, Camarillo, Calif) according to the manufacturer's instructions. The minimum detectable doses for individual cytokines are as follows: 8.7 pg/mL for IL-2, 2.0 pg/mL for IL-4, 5.0 pg/mL for IL-10, and 4.0 pg/mL for IFN-γ.

Statistical analysis

To determine whether the method of administration of the rAds (abrasion and injection) and the analysis of the immune responses at various time periods after administration (days 0, 3, 22, 31, 49, etc.) influenced the Ad-specific proliferative responses, mixed-factors analysis of variance techniques were used. The Student *t* test was used to assess changes in IL-10 production.

RESULTS

Recently, we have described the successful insertion of the Ad5CMV-β-gal gene into the cervix of rhesus monkey by direct injection as opposed to the abrasion technique.[12] In these monkeys, we also observed the induction of systemic Ad-specific humoral immune responses.[12] In the present study, we analyzed the Ad-specific cellular immune responses induced in these monkeys. To assess the Ad-specific proliferative responses, PBMCs were incubated with either purified virus or virus-infected 293 cell extract as test Ag and with culture medium or uninfected 293 cell extract as a control Ag.

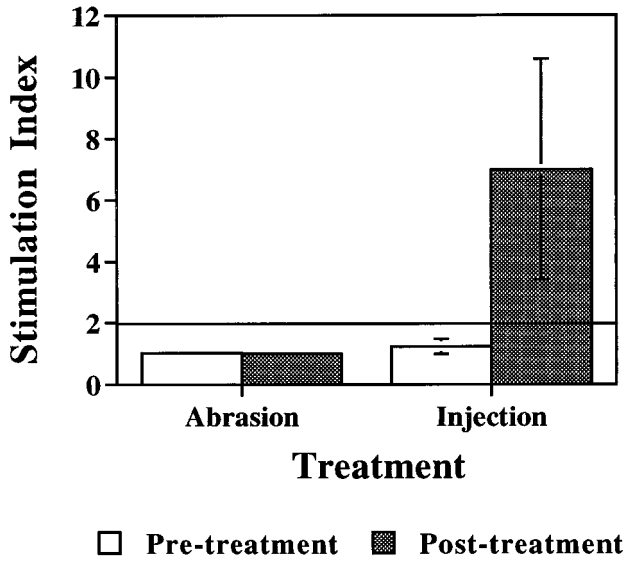

Figure 1. Comparison of Ad-specific proliferative responses in rhesus monkeys subjected to either the abrasion or direct injection technique for administration of the rAds. PBMCs from monkeys receiving adenovector by abrasion (a total of 5) or injection (a total of 10) were incubated with extracts from either control or Ad-infected cells. Proliferative responses, in terms of SI, were measured by [^{3}H]thymidine incorporation assay before and at 7–8 weeks after insertion of the rAd. See *Materials and Methods* for experimental details.

Introduction of rAd into the cervix of rhesus monkeys by direct injection but not abrasion results in induction of specific proliferative responses

The Ad-specific proliferative responses induced in two separate groups of rhesus monkeys that received the rAd Ad5CMV-β-gal in the cervix by either the abrasion technique or direct injection were compared; the data are presented in Figure 1. Neither group of monkeys exhibited Ad-specific proliferative responses before treatment with the rAd. However, it is clear from the data collected at 7–8 weeks posttreatment that significant proliferative responses were primed in the group of monkeys that received the rAd by direct injection but not in those subjected to the abrasion technique ($F(1,13) = 9.26$, $P \leq .01$). These results are consistent with our previous observation that introduction of Ad5CMV-β-gal into the cervix by direct injection but not by the abrasion technique results in the expression of β-gal in the cervix of rhesus monkeys.[12]

Higher doses and multiple treatments by the abrasion technique to introduce Ad5CMV-β-gal into the cervix of rhesus monkeys fail to prime specific proliferative responses

The data from the analysis of the proliferative responses at different timepoints after the introduction of the rAd by the abrasion technique in the five monkeys are shown in Table 1. There was no significant difference in proliferative responses in terms of SI values between base-level samples and samples obtained at 3 days posttreatment in all of the monkeys (SI <2). An additional dosing of Ad5CMV-β-gal by a second abrasion on day 28 also showed no significant levels of rAd-specific proliferative responses (SI values at day 49 were less than the positive cutoff value of 2.0, Table 1), indicating the absence of a memory immune response. Another observation in these monkeys is that, despite using increasing doses of the rAd (2×10^8 to 2×10^{10} PFU), none of the monkeys showed positive Ad-specific proliferative responses. These results paralleled our previous observation that introduction of the rAd at doses of $\leq 2 \times 10^{10}$ PFU into the cervix of rhesus monkeys by the abrasion technique does not result in transgene expression.[12]

Induction of proliferative responses do not depend upon the dose of the rAd used for injection into the cervix of the rhesus monkey

The data from the analysis of the proliferative responses (SI) at different timepoints after the injection of Ad5CMV-β-gal in the cervix of the monkeys (group II, comprising monkeys L571, L741, L87, L345, L577) are shown in Table 2. No Ad-specific proliferative responses were observed at 3 days after injection of the rAd in these monkeys (SI <2), even though significant PHA-specific responses were present in all of these monkeys (the Δ counts per minute (cpm) and SI values ranged from 2205 to 26578 and from 9.5 to 198.3, respectively). There was no significant difference in Ad-specific proliferative responses between day 0 and day 3 ($F(1,4) = 0.16$, $P > .05$). Alternatively, higher proliferative responses, compared with those at day 0, were observed on days 22 ($F(1,2) = 17.30$, $P \leq .05$), 49 ($F(1,4) = 14.70$, $P \leq .05$), and 83 ($F(1,4) = 20.30$, $P \leq .01$) postinjection. In this group, monkey L571, which received Ad5CMV-β-gal at 2×10^8 PFU, showed an SI of 18.9, whereas monkey L87, with an injected dose of 2×10^9, showed an SI of 45, indicating a 2.3-fold increase in proliferative response with the increased dose. However, a further increase in the dose to 2×10^{10} in monkey L345 did not result in enhancement of the response, indicating the lack of a strong correlation between injection dose and the level of Ad-specific proliferative responses. Two additional injections of Ad5CMV-β-gal to the animals in this group on days 25 and 56, at the same dose as the original injection, did not influence the levels of Ad-specific proliferative responses: the SI values ranged between 2.1 and 8.0 on day 49, and those on day 83 were between 2.2 and 5.1, indicating that adenovector administration did not affect the normal cellular immunity. The PHA-specific proliferative responses were also unaltered in all of these monkeys at these timepoints (the SI values ranged between 5.8 and 181.2 and between 9.2 and 160.3 on days 49 and 83, respectively). Overall, these results suggest that a single injection of Ad5CMV-β-gal is sufficient to induce a strong proliferative response to Ad, and that additional injections or higher doses do not have enhancing effect on the level of response. Whereas significant Ad-specific proliferative responses were observed for ≤83 days in these monkeys, analysis at day 181 showed only base-level values (SI <2).

Analysis of the transgene expression in the tissue sections at different time periods after the rAd Ad5CMV-β-gal had been injected into the cervix of the monkeys revealed high levels of β-gal expression only at 3 days after primary injection (Fig 2A). At this time, no proliferative responses specific to the rAd were observed (Table 2). Alternatively, no transgene expression was observed at the 22-day and 49-day timepoints (Fig 2, B and C, respectively), when the PBMCs isolated from these monkeys exhibited significant levels of Ad-specific proliferative responses (Table 2). These results indicate an inverse relationship between transgene expression and rAd-specific proliferative responses. Further, these data suggest that although introduction of the rAd into the cervix of the rhesus monkey is effective for an efficient induction of transgene expression, it also primes strong systemic cellular immune responses in the host that abrogate the sustained expression of the transgene in the cervix. A similar experiment with an additional group of five monkeys (group III) was conducted to confirm these results.

The five monkeys in group III were treated with equal amounts of two different rAd constructs, Ad5CMV-β-gal and Ad5CMV-p53 (1×10^{10} PFU each), by direct injection into the cervix. The data from the analysis of the proliferative responses at different timepoints postinjection are presented in Table 3. Similar to the animals in group II, these monkeys also did not exhibit Ad-specific proliferative responses on day 3 following injection of the rAds. A comparison of SI values between day 0 and day 3 showed no significant difference ($F(1,4) = 3.90$, $P > .05$). However, these monkeys showed strong proliferative responses to PHA both on day 0 (Δ cpm and SI values ranged from 8851 to 10998 and from 8.5 to 23.8, respectively) and day 3 (Δ cpm and SI values ranged from 4788 to 28729 and from 3.8 to 116.5, respectively). Analysis on day 31 revealed significantly higher levels of Ad-specific proliferative responses than on day 0 in all five monkeys in this group, as evidenced by SI values that ranged from 5.7 to 26.1 ($F(1,4) = 21.32$, $P \leq .01$). Also, higher Ad-specific proliferative responses were observed up to day 59 ($F(1,4) = 8.33$, $P \leq .05$) in all of the monkeys in this group; these responses decreased to insignificant levels after 155 days ($F(1,4) = 0.30$, $P > .05$). However, the Ad-specific proliferative responses observed in the group III monkeys after a total of three injections with a mixture of the two different constructs (Ad5CMV-β-gal and Ad5CMV-p53) were not significantly different from

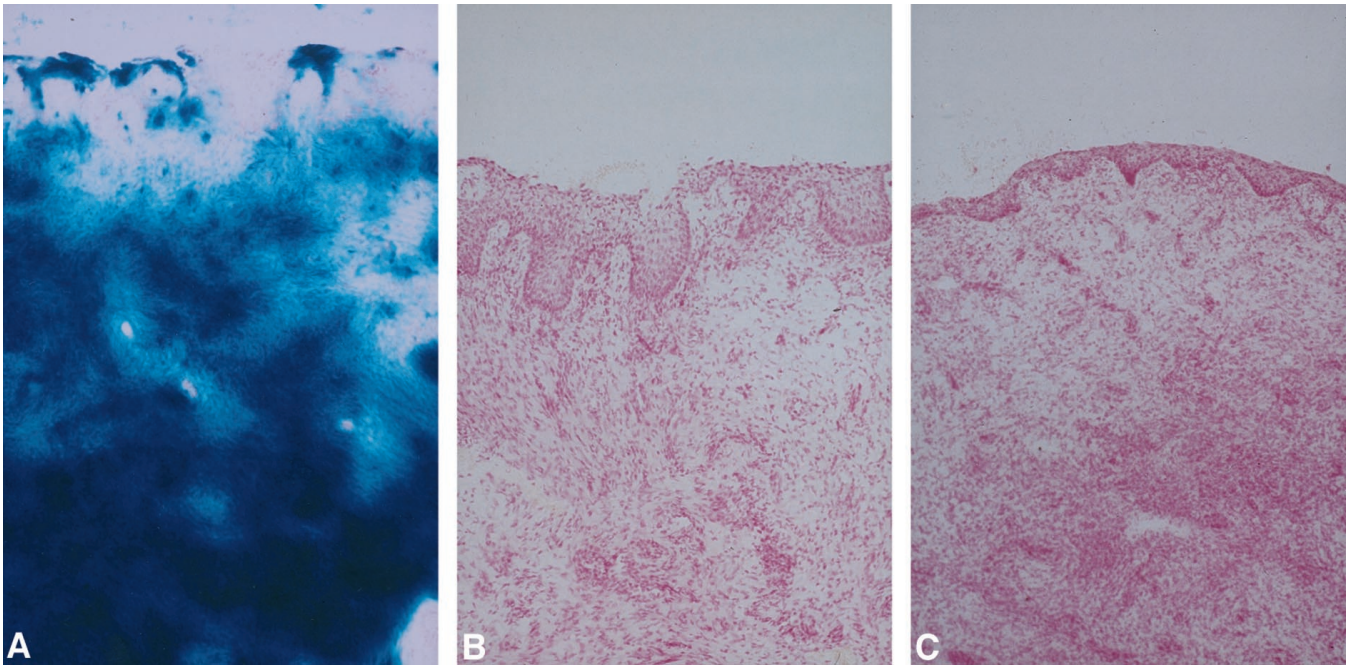

Figure 2. Transgene expression in the rhesus monkey cervix after injection with 2×10^{10} PFU of Ad5CMV-β-gal (original magnification is $\times$10). The β-gal stain is present in stroma staining roughly all cells in a biopsy taken 3 days postinjection **(A)** but not in the samples taken after 22 **(B)** and 49 **(C)** days.

the responses observed in the monkeys in group II that received only Ad5CMV-β-gal ($F(1,6) = 0.02$, $P > .05$).

Similar to the results from the group II monkeys, an analysis of transgene expression in the tissue biopsy samples from the cervix of the animals in group III also revealed only transient expression of the transgene (both β-gal and p53), which was abrogated concomitant with the induction of the Ad-specific proliferative responses (data not shown). Overall, data from the 10 rhesus monkeys indicate that the introduction of rAds into the cervix by direct injection results in the induction of strong systemic Ad-specific proliferative responses that adversely effect the sustained transgene expression.

Cytokine production by PBMCs from rhesus monkeys following injection of rAd in the cervix

The data from the two groups of rhesus monkeys showing consistent priming of the Ad-specific proliferative responses subsequent to injection of the rAds into the cervix prompted us to further characterize the Ad-specific cellular immune responses in these monkeys. Toward this goal, we analyzed the cytokine production by PBMCs from three monkeys each in groups II and III in response to *in vitro* stimulation with the Ad Ag. Of the five different cytokines assayed (IL-2, IL-4, IL-10, IL-12, and IFN-γ), we observed significant production of only IL-10 in four monkeys. The data showing the levels of IL-10 and the corresponding proliferative responses (in terms of SI) on various days following injection in six monkeys are presented in Figure 3. In monkeys L571, L741, L345, and L515, we observed the production of significant levels of IL-10 (fold increase compared with background values ranged from 15 to 54) by the day 3 after the first injection ($t = 10.15$, degrees of freedom = 3, $P \leq .01$). Although a slight increase in IL-10 levels was observed on day 25 in the monkey L571, there was a steady decline over time that reached negligible levels between days 155 and 205 in all monkeys. No significant production of IL-10 was observed in monkeys J23 and L559 at any of the timepoints tested.

DISCUSSION

Adenovector-mediated gene therapy offers an attractive approach to introduce genes *in vivo*. To the best of our knowledge, no human clinical trials have yet been attempted for Ad-mediated gene therapy of cervical cancer. However, our previous studies with adenovector-mediated delivery of p53 or antisense constructs corresponding to the E6/E7 oncogenes of HPV-16 to human cervical cancer cell lines *in vitro* and in mouse experiments have shown the tremendous potential of exploring this approach for cervical cancer.[11,18] Further, we demonstrated the feasibility of direct insertion of the adenovector into the monkey cervix.[12] Our studies in the nonhuman primate model have also shown an induction of humoral immunity to the rAd.[12] The present study provides further insight into the immune mechanisms affecting transgene expression subsequent to direct introduction of the rAds into the cervix of the rhesus monkey.

Our results clearly showed the priming of significantly

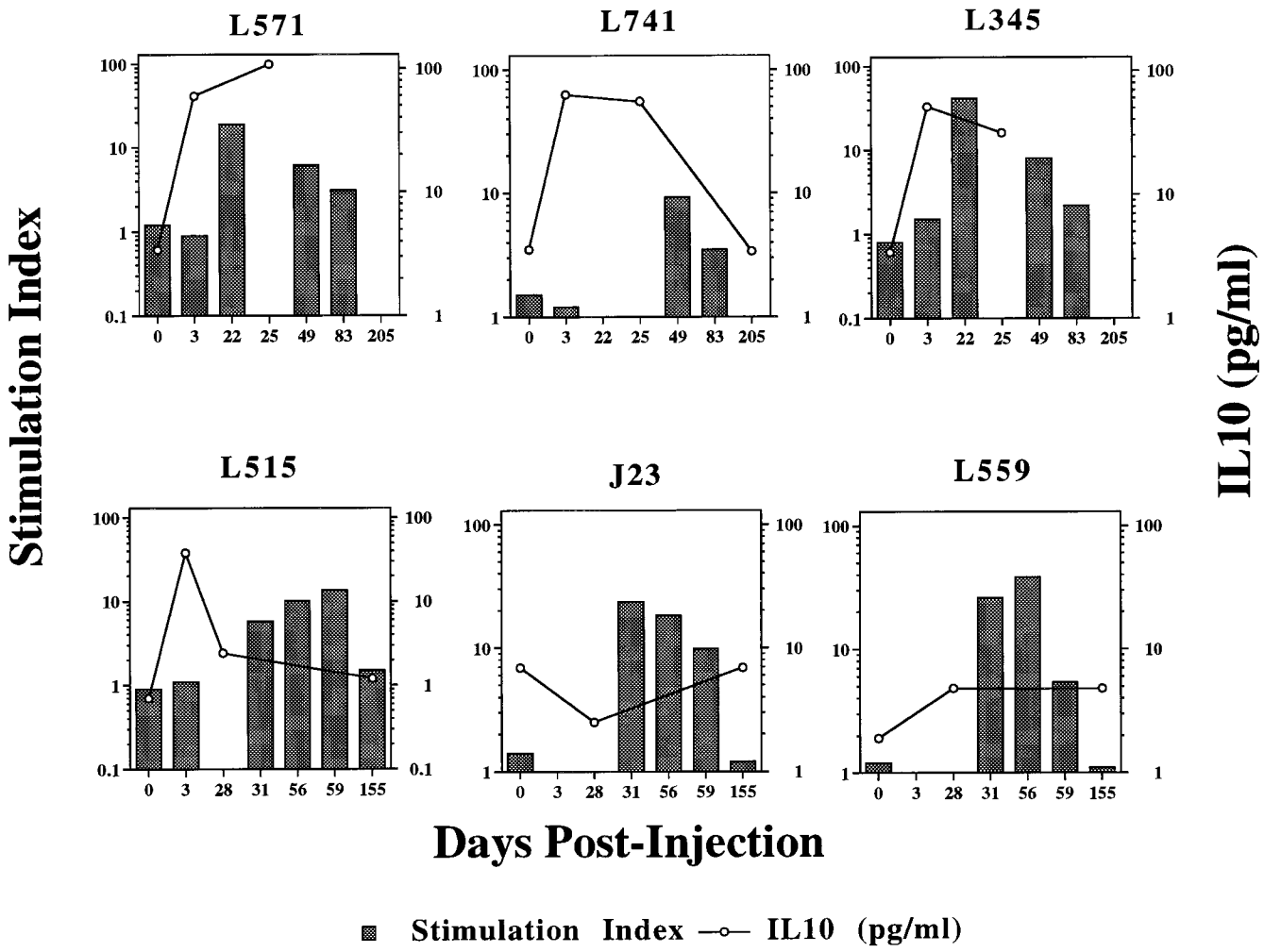

Figure 3. Ad-specific proliferative responses and IL-10 production by PBMCs from monkeys injected with rAd. Rhesus monkeys were injected with Ad5CMV-β-gal alone (L571, L741, and L345) or with Ad5CMV-β-gal in combination with AD5CMV-p53 (L515, J23, and L559) in the cervix; the PBMCs isolated from the blood samples obtained at various timepoints were incubated with either virus or medium. The proliferative response, in terms of SI, was measured by [^{3}H]thymidine incorporation, and the amount of IL-10 produced was measured by enzyme-linked immunosorbent assay. See *Materials and Methods* for experimental details.

high levels of specific proliferative responses in rhesus monkeys with rAds (either Ad5CMV-β-gal alone or a combination of Ad5CMV-β-gal and Ad5CMV-p53) by direct injection but not by the abrasion technique into the cervix. Analysis of the tissue biopsy samples obtained from the cervix of these animals revealed only transient expression of the transgene (β-gal and/or p53), which was abrogated concomitant with the induction of the Ad-specific proliferative responses. These results correlate well with our previous data, which demonstrated efficient induction of Ad-specific immunoglobulin G in only those monkeys that were subjected to injection to introduce the rAd.[12] In addition to the specific proliferative responses, we also observed the production of IL-10, which is a cytokine that is characteristic of T helper type 2 (Th2) cellular immune responses. The presence of significant levels of IL-10 preceded the priming of rAd-specific proliferative responses. These results, together with the observations regarding the level and longevity of both the humoral and cellular immune responses, strongly suggest an immunological basis for the failure of long-term transgene expression in these monkeys that are subjected to rAd-mediated gene transfer.

Our results clearly demonstrated efficient transgene expression in the cervix at 3 days after primary injection with the rAds. At this time, analysis of the blood samples showed no Ad-specific proliferative responses in any of these animals. The failure of transgene expression paralleled the sustained high levels of both humoral and cellular immune responses that were observed starting at day 22 (see data in Tables 1, 2, and 3, and Ref. 12). Taken together, these results clearly demonstrate not only the high immunogenicity of the rAds used in our study but also significant differences between the kinetics of transgene expression and the priming of immune

responses. Because our experimental protocols did not involve either repeated sampling or repeated dosing within 3 weeks of primary injection of the rAd into the cervix, we do not know whether transgene expression could be sustained beyond 3 days. Because our gene therapy approach against cervical cancer is based on efficient *in situ* expression of wild-type p53, and because sustained expression of p53 for short periods may be sufficient for a favorable outcome, our observations of the differential kinetics of transgene expression and the induction of anti-Ad immunity offer possibilities for future modifications and further development of this approach. In this regard, we observed the production of significant levels of IL-10 by PBMCs isolated from four different monkeys at 3 days after injection with the rAds, even though strong transgene expression was evident at this time.[12] Because the production of IL-10 represents a typical Th2 cellular immune response, and because Th2 responses promote the development of efficient humoral immune responses, curtailing the IL-10 production that seems to precede both humoral and cellular immune responses[19,20] may be another viable option for improving our gene therapy approach.

Whereas the rhesus monkeys used in our studies may be naive with respect to the Ad, the majority of the human population is exposed to some strain of Ad sometime during their lifetime. However, Bramson et al[21] reported recently that the preexisting immunity to Ad in mice, by virtue of prior immunization, did not prevent tumor regression following an intratumoral administration of recombinant adenoviral vector encoding IL-12. Actually, in this model, the preexisting immunity reduced virus dissemination to other organs such as the liver, which was suggested to be advantageous. In any case, the preexisting immunity to Ad or that induced following adenovector administration could be modulated by the use of immunosuppressive drugs to achieve long-term transgene expression. In this regard, preclinical studies in the murine model have shown that the administration of cyclophosphamide[22] or etoposide,[23] the latter of which is a widely used immunosuppressive drug in cancer patients, before adenovector injection diminished both cellular and humoral responses to Ad and enabled long-term transgene expression. Our future studies will include the use of such immunosuppressive reagents to extend the window of transgene expression in the cervix to a time period that is sufficient to achieve the desired therapeutic endpoint.

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