IMMUNOLOGICAL ASPECTS OF THE INTERVERTEBRAL DISC: AN IN-VIVO STUDY OF ADENOVIRUS-MEDIATED GENE TRANSFER

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INTRODUCTION
Adenoviral vectors can efficiently deliver genes to a wide variety of dividing and non-dividing cell types. However, in most tissues/organs, current adenoviral vectors are able to facilitate only short-term in-vivo transgene expression (1-2 weeks) because they elicit strong immune responses by the host (1). The intervertebral disc appears to be an exception: adenovirus-mediated marker gene transfer to the rabbit intervertebral disc has previously been reported to result in remarkably long-term in-vivo gene expression (up to 12 weeks) (2,3). The results of this study were obtained in a rabbit (4, Figure 1B) from the immunized group (Group B) in spite of a high levels of neutralizing antibody production. Intradiscal luciferase activity alone was not sufficient to provoke an immune response—as evidenced by the antibody levels in two rabbits (5 and 6, Figure 1A) from the non-immunized group (Group A).

METHODS
Surgical procedures and in-vivo transduction. Eight skeletally mature New Zealand white rabbits, 4-5kg each, were used in this study. The anterior aspects of the lumbar intervertebral discs were exposed using a retroperitoneal approach. In four rabbits (Group A), 15 μl of saline containing Ad/CMV-luciferase particles (6x10^6 plaque forming units (PFU)) were injected through a 28-gauge hypodermic needle into the nucleus pulposus of the L2-3 and L3-4 intervertebral discs. In remaining four rabbits (Group B), 15 μl of saline with Ad/CMV-luciferase (6x10^6 PFU) were injected into the L2-3 and L3-4 intervertebral discs, and, in addition, 6x10^6 PFU of adenovirus construct diluted in 1 ml of saline was injected at multiple sites into subcutaneous tissues for immunization. After injection, all wounds were closed routinely. Detection of antibodies to adenovirus. Blood samples were harvested from the ear artery before surgery and 3, 7, 14, 21, and 42 days after surgery. The serum was separated and these samples were stored at -80°C until the time of assay. Ninety-six well MaxiSorp immunoplates (Nunc) were coated with 1x10^5 PFU Ad/CMV-Luciferase in 100 μl phosphate buffered saline (PBS) overnight at 4 °C. The wells were thoroughly rinsed three times with PBS containing 0.05% Tween-20 (Sigma), and the protein binding sites were blocked by non-fat skin milk (200 μl) incubated for 1 hr. at room temperature (RT). 1:100 dilutions of rabbit serum in 100 μl PBS containing 0.05% Tween-20 (Sigma) and 1% phosphate substrate (Sigma) in PBS for 20-30 min at RT. Optical densities (O.D.) were measured at 405 nm. Luciferase Assay. Six weeks after surgery, the rabbits were sacrificed. To evaluate intradiscal transgene expression quantitatively, luciferase gene expression in harvested nucleus pulposus tissues was assessed using a standard luciferase assay kit (Promega). Light production over a period of 30 seconds was measured with a luminometer, and expressed in relative light units (RLU).

RESULTS
No rabbits developed systemic illness secondary to virus injection. At the time of sacrifice, almost all discs appeared macroscopically normal, but some demonstrated spur formation at the site of injection. Humoral Immune Response. Prior to intradiscal injection of Ad/CMV-luciferase, no rabbits possessed neutralizing antibodies to adenoviral proteins above background levels. After injection, all rabbits in the immunized group (Group B) exhibited significantly increased production of neutralizing antibody within 2 weeks of injection (Figure 1B). In contrast, there was a variable amount of neutralizing antibody production in the rabbits of Group A: two rabbits exhibited increased production of antibody within 3 weeks of injection, while the remaining two exhibited little or no increase in antibody production (Figure 1A).

Intradiscal transgene expression. Six weeks after intradiscal injection of Ad/CMV-luciferase, the disc tissue from all of the rabbits exhibited luciferace activity. The highest levels of intradiscal luciferace activity in this study was obtained in a rabbit (4, Figure 1B) from the immunized group (Group B) in spite of a high levels of neutralizing antibody production. Intradiscal luciferase activity alone was not sufficient to provoke an immune response—as evidenced by the antibody levels in two rabbits (5 and 6, Figure 1A) from the non-immunized group (Group A).

DISCUSSION
The results of this study support our hypothesis that the intervertebral disc is an immunologically-privileged site. The differences between the Group A and Group B humoral immune responses suggest that adenovirus may have leaked from the discs of two rabbits from Group A following intradiscal injection, resulting in the “priming” of their immune systems. Nevertheless, significant levels of transgene expression occurred in all discs up to 6 weeks following gene transfer—suggesting that the disc limits access of antibodies to the nucleus pulposus.

Future investigations will be aimed at characterizing the maximum length of intradiscal transgene expression in presence of neutralizing antibodies, and determining whether there is a delayed immune response to viral antigens.

REFERENCES

ACKNOWLEDGMENTS
The authors thank Dr. Savio L-Y. Woo, Dr. Freddie H. Fu, and Dr. Kosaku Mizuno for generous guidance and support of this study.