Development of Adenovirus Vectors Encoding Rat Complement Regulators for Use in Therapy in Rodent Models of Inflammatory Diseases

Yvonne McGrath, Gavin W. G. Wilkinson, O. Brad Spiller and B. Paul Morgan

*J Immunol* 1999; 163:6834-6840; http://www.jimmunol.org/content/163/12/6834

**References**  This article cites 46 articles, 14 of which you can access for free at: http://www.jimmunol.org/content/163/12/6834.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Development of Adenovirus Vectors Encoding Rat Complement Regulators for Use in Therapy in Rodent Models of Inflammatory Diseases

Yvonne McGrath,* Gavin W. G. Wilkinson,† O. Brad Spiller,* and B. Paul Morgan‡*‡

C activation has been implicated in the pathogenesis of numerous inflammatory human diseases and disease models. A therapy based on C inhibition might therefore be of benefit to reduce inflammation and ameliorate disease. C inhibition in vivo can be accomplished by the delivery of soluble recombinant C regulators either systemically or directly to a target site, but effects are transitory. We have developed a strategy for the efficient delivery of the membrane-bound rat C inhibitors, CD59, Crry, and decay-accelerating factor (DAF), using replication-deficient adenovirus vectors with the intention of treating rat models of disease in which C is implicated. The adenovirus recombinants (RAd), RAdCD59, RAdCrry, and RAdDAF, respectively, have been tested for expression and function of the transgene in vitro. Infection of human fetal foreskin fibroblasts resulted in high levels of expression of each of the rat inhibitors. The constructs were also tested for inhibition of rat C-mediated cell lysis and C3b deposition. In a cell lysis assay, each inhibited to varying degrees of efficiency in the order RAdCD59 > RAdDAF > RAdCrry. In a C3b deposition assay, RAdDAF caused a greater reduction in C3b deposition than RAdCrry and RAdCD59 was ineffective. These agents, individually or in combination, provide the tools for testing the effects of prolonged inhibition of C at a target site on the progress of experimental models of disease. The Journal of Immunology, 1999, 163: 6834–6840.


The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

3 Abbreviations used in this paper: DAF, decay-accelerating factor; GPI, glycosylphosphatidylinositol; PIPLC, phosphatidylinositol-specific phospholipase C; MCF, mean cell fluorescence; MCP, membrane cofactor protein; p.i., postinfection; MOI, multiplicity of infection; SCR, short consensus repeat; VBS, Veronal-buffered saline; RAd, recombinant adenovirus; HFFF, human fetal foreskin fibroblast; β-gal, β-galactosidase.
C. Successful delivery of a transgene to rat synovium was demonstrated by intrarticular injection of an adenovirus recombinant encoding *Escherichia coli* β-galactosidase (β-gal).

Materials and Methods

**Abs and cDNAs**

Mouse anti-rat CD59 mAb (6D1) and mouse anti-rat DAF mAb (RDIII7) were raised in house and are described elsewhere (17, 25). 6D1 and RDIII7 Abs do not cross-react with their human counterparts. Cry polyclonal antisera was used in rabbits by standard techniques. Anti-rat Cry mAb TLD1C11 was a gift of Dr. W. F. Hickey (Dartmouth College, Hanover, NH). Mouse anti-human C3c mAb (C3/50), cross-reactive with rat C3c, was a gift of Mr. Peter Taylor (Norvartis, Horsham, U.K.). To sensitize human cells to C attack, antisera to a subclone of the U937 cell line, which does not express CD59, was raised in rabbits (26). The serum was tested by immunoblotting and found not to cross-react with rat C inhibitors. Antiserum was incubated at 56°C for 30 min to inactivate C. Human-anti-rat C5d9 (Bric-229) was purchased from the International Blood Group Reference Laboratory (Bristol, U.K.). Anti-human DAF (1H4) was a gift from Dr. W. F. Hickey (Dartmouth College, Hanover, NH). CD59 polyclonal antibody was raised in rabbits by standard techniques. Anti-rat C3c and anti-rat DAF mAbs do not cross-react with their human counterparts. RAd were plaque purified, grown to high titer in 293 cells as described previously (31), and extracted using an equal volume of Arklone P (Basic Chemical Company, High Wycombe, U.K.). The virus was further purified by cesium chloride gradient ultracentrifugation and dialysis against 1 mM MgCl₂, 135 mM NaCl, 10 mM Tris–HCl (pH 7.8), 10% glycerol (31). Titters were determined by standard plaque assay in 293 cells (32). Three viruses were generated: RAdCD59 which encodes GPI-anchored rat CD59; RAdDAF which encodes GPI-anchored rat DAF; and RAdCry encoding the seven SCR (short consensus repeat) form of transmembrane rat Cry. The fidelity of the C regulator sequences in each of the viruses was confirmed by sequencing.

**Testing expression in HFFFs by immunofluorescence**

HFFs were grown to 80% confluence on coverslips, and infections were set up in a minimum amount of medium. A crude Arklon e extract of the third plaque purification was used for immunofluorescence microscopy to test for expression of the C regulator before large scale production of the virus. Cells were infected for 4 h at 37°C at which time the medium was replaced. After a further incubation for 16 h at 37°C, the cells were washed in PBS, dried, and fixed in methanol-acetone (1:1) for 30 s. Cells were incubated with 10 μg/ml CD59 mAb 6D1, Cry mAb TLD1C11, or DAF mAb RDIII7 for 30 min at 37°C, washed, and detected by incubating with 10 μg/ml goat anti-mouse FITC conjugate at 37°C for 30 min.

**Testing for expression in HFFFs by Western blot analysis**

HFFs were infected in 35-mm dishes at a multiplicity of infection (MOI or number of PFU per cell) of 100 as described above. Four hours postinfection (p.i.) the cells were washed in PBS and overlaid with serum-free medium. Cells lysates were prepared day 3 p.i. by resuspending cells in 100 μl Townsend lysate buffer, vortexing, and incubating on ice for 15 min. Cell lysates were stored at −70°C in nonreducing SDS-PAGE buffer (Bio-Rad) until use. Uninfected cells were used to detect nonspecific binding of either the primary or secondaryAbs. RAdCD59-infected cell lysates were run on a 15% SDS-PAGE nonreducing gel and RAdDAF- and RAdCry-infected cell lysates on a 10% nonreducing gel before blotting onto nitrocellulose membranes (Sartorius, Göttingen, Germany). After transfer, blots were blocked in 5% nonfat milk powder in PBS for 30 min at room temperature and then probed with CD59 mAb 6D1, Cry mAb TLD1C11, or DAF mAb RDIII7 at 1 μg/ml for 1 h at 4°C. Blots were washed in PBS, 0.1% Tween 20 and incubated for 1 h at 4°C with 1 μg/ml goat anti-mouse HRP conjugate. Development was with the ECL detection kit (Amersham, Little Chalfont, U.K.) according to the manufacturer’s instructions.

**Flow cytometric analysis and PIPLC cleavage of the GPI anchor**

Cells were infected in six-well trays at 80% confluency at MOIs of 50 and 500 in a minimum amount of medium. Medium was changed after 4 h, and cells were cultured for a further 3 days. Cells were detached with flow cytometry solution (15 mM EDTA, 30 mM NaCl, 1% BSA in PBS), and protein expression was detected by incubating 10⁶ cells with 5 μg/ml primary Ab for 30 min at 4°C. CD59 mAb 6D1, Cry polyclonal antiseraum and DAF mAb RDIII7 were used to detect CD59, Cry, and DAF, respectively. For all three viruses, a control Ab, OX23, was used to quantify nonspecific Ab binding due to adenovirus infection. The cells were washed in flow cytometry solution, and the primary Ab was detected with a goat anti-mouse or anti-rabbit PE conjugate at 4°C for 30 min. The cells were washed, the signal detected on a Becton Dickinson FACScalibur flow cytometer (Heidelberg, Germany) and analyzed using Cell Quest software (Becton Dickinson). To assess the integrity of the CD59 and 1GPI anchor, the ability of phosphatidylinositol-specific phospholipase C (PIPLC, Roche, Lewes, U.K.) to release the protein from the membrane was assessed. Cells (10⁶) infected at an MOI of 100 with RAdCD59, RAdCry, or RAdDAF were either treated with PIPLC at 1/100 for 45 min at 37°C or incubated without PIPLC. Cells were then washed and stained for FACs analysis as described above.

**C3 deposition assays**

Pilot studies were used to determine optimum serum and sensitizing Ab concentrations for maximum C3 deposition with minimum cell lysis on uninfected cells. HFFs were infected with RAd either singly or in combination with MOIs of 100 and 300 or left uninfected. On day 3 p.i., the cells were left unsensitized and were incubated with a 1/5 dilution of rat serum in PBS for 30 min at 37°C. Negative controls were performed using VBS, heat-inactivated serum, or serum containing 10 mM EDTA. The cells
were washed in PBS and detached in flow cytometry solution. C3 deposition was detected with anti-C3c mAb (C3/30 ascites at 1/500) by flow cytometry as described.

Results were analyzed by one-way ANOVA. Posttests were done on relevant groups of data and Bonferroni corrected for multiple comparisons.

Cell lysis assays

HFFFs in 24-well plates were infected at MOIs of 100, 200, and 300 with RAdCD59, RAdCrry, and RAdDAF either singly or in combination or left uninfected. On Day 3 p.i., the cells were loaded with calcein-AM (Molecular Probes, Eugene, OR) at 2 μg/ml in MEM medium (including supplements) at 37°C for 1 h. The medium was aspirated off, and the cells were sensitized with 100 μl/ml of rabbit anti-IgG serum in Hanks’ medium for 15 min at 37°C. During sensitization, human CD59 and DAF were blocked on the HFFFs using 10 μg/ml of Bric-229 and IH4. Blocking and sensitization had previously been shown to increase rat serum-induced lysis. Cells were washed and exposed to C attack with rat serum diluted to 1/5 in VBS. Supernatant was removed completely after 30 min at 37°C, and the remaining unlysed cells were lysed by addition of 0.1% Triton X-100 in water. The lysate was saved, and calcein was measured in the supernatant and lysate on a Wellfluor fluorometer (excitation filter wavelength, 485 nm; emission filter wavelength, 530 nm). Percentage of cell lysis was calculated as [calcein release by serum/calcein release by serum + calcein release by detergent] × 100.

Results were analyzed by one-way ANOVA. Posttests were done on relevant groups of data and Bonferroni corrected for multiple comparisons.

In vivo delivery of RAd to rat synovium

PVG-C rats were used for in vivo delivery experiments. Rats were anesthetized, and 3.5 × 10⁸ PFU of RAd35 were injected intraarticularly into the right hind knee joint. Control animals were mock-injected. On day 3 postinjection, the rats were sacrificed, and the patella and surrounding tissue were dissected off both the experimental and control knees. The tissue was frozen in CRYO-M-BED (Bright Instrument, Huntingdon, Cambridgeshire, U.K.), and 12-μm sections were cut on a cryostat (Bright Instrument). Sections were fixed in 0.5% glutaraldehyde and stained in 3 mM potassium ferrocyanide, 3 mM potassium ferricyanide, 1.3 mM MgCl₂, and 130 μg/ml 5-bromo-4-chloro-3-indolyl-β-n-galactoside for 2 h at room temperature before viewing.

Results

Expression of rat C inhibitors on human fibroblasts

Expression of rat CD59, Crry, and DAF on HFFFs infected with the relevant recombinant virus was first demonstrated by immunofluorescence microscopy (Fig. 1). As is typical for adenovirus expression in human fibroblasts, the expression was heterogeneous, with some cells showing much higher expression than others. This heterogeneity can be accounted for by the dependence of the CMV promoter strength on the cell cycle stage of the host cell.

Background staining on uninfected cells for all three Abs was negligible; a typical example is shown in Fig. 1D. Western blot analysis was used to confirm that the recombinant proteins were of the correct size as compared with the native protein expressed on rat erythrocytes (Fig. 2). Rat CD59 in the RAdCD59-infected cells comigrated with the major erythrocyte CD59 band at ~18–20 kDa as expected (Fig. 2A). Rat DAF in the RAdDAF-infected cells ran as a band of ~65 kDa, which correlates well with that seen in the rat erythrocyte lane (Fig. 2C). A higher m.w. species of ~120 kDa was also seen in the lanes positive for DAF, which represents DAF dimers. Rat Crry in the RAdCrry-infected cells ran as a single band of 65–68 kDa, whereas rat erythrocytes contained bands of 55 and 65 kDa, corresponding to the 6 SCR and 7 SCR forms of Crry found on rat erythrocytes (Fig. 2B) (20, 22). All uninfected control lysates were consistently negative. By flow cytometry, it was apparent that each of the regulators was expressed at a high level on infected cells (Fig. 3). The level of expression was proportional to

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** Immunofluorescence microscopy demonstrating the expression of rat C inhibitors. HFFFs were infected with RAdCD59 (A), RAdCrry (B), and RAd DAF (C), and transgene expression was detected with the primary mAbs 6D1, TLD1C11, and RDIII7, respectively. Secondary detection was with goat anti-mouse FITC conjugate. Negative controls were performed by staining uninfected cells. The uninfected cells shown in D are stained with 6D1 and represent a typical negative result.

**FIGURE 2.** Size comparison of recombinant rat C inhibitors to endogenous by Western blot analysis. HFFFs were infected with RAdCD59 (A), RAdCrry (B), and RAdDAF (C). Cell lysates were prepared 3 days p.i. and run alongside rat erythrocyte ghosts as positive controls. Cell lysates of uninfected cells were run as negative controls. Detection of CD59, Crry, and DAF was with the primary mAbs 6D1, TLD1C11, and RDIII7, respectively. Goat anti-mouse HRP conjugate was used as the secondary Ab.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** Size comparison of recombinant rat C inhibitors to endogenous by Western blot analysis. HFFFs were infected with RAdCD59 (A), RAdCrry (B), and RAdDAF (C). Cell lysates were prepared 3 days p.i. and run alongside rat erythrocyte ghosts as positive controls. Cell lysates of uninfected cells were run as negative controls. Detection of CD59, Crry, and DAF was with the primary mAbs 6D1, TLD1C11, and RDIII7, respectively. Goat anti-mouse HRP conjugate was used as the secondary Ab.
an increasing MOI up to an MOI of 500. Fig. 3 shows representative traces at a MOI of 50 and 500 to illustrate this point. At a MOI of 30, almost 100% of the cells expressed the transgene, and the dose-dependent increase in expression above this MOI is likely due to multiple infection of individual cells. No further increase in expression could be demonstrated above MOIs of 500 for any of the recombinants. Because the Ab was not limiting, this ceiling on expression was probably due to the saturation of the expression machinery of the cells. Indeed at the highest MOIs, we observed virus-induced cytotoxicity, manifesting as holes in the cell monolayer. In vitro expression levels were followed for 7 days by flow cytometry. Expression levels peaked at day 3 but remained high at day 7 p.i. (data not shown). No increase in binding of the control mAb OX23 was seen in any infection at any MOI (results not shown).

Release of CD59 and DAF by PIPLC demonstrates the integrity of the anchor

A 69% reduction in mean cell fluorescence was demonstrated on treatment of RAdCD59-infected cells with PIPLC (Fig. 4A). Similarly, DAF showed a 57% reduction in mean cell fluorescence after PIPLC treatment (Fig. 4B). In contrast, the mean cell fluorescence for Crry actually increased by 71% after PIPLC treatment.

Expression of rat C inhibitors by RAd infection inhibits C3 deposition on human fibroblasts

RAdCrry significantly inhibited C3 deposition on human fibroblasts in a dose-dependent manner at MOIs 100, 200, and 300 over uninfected controls (p < 0.01, p < 0.01, and p < 0.001, respectively) (Table I). Similarly for RAdDAF, significant and dose-dependent inhibition of C3 deposition was seen at all three MOIs (p < 0.001 for each comparison). RAdCrry, although less effective, also offered a significant degree of protection over control virus (p < 0.001 for each comparison). RAdCrry, as expected, did not inhibit C3 deposition on human fibroblasts at any MOI. Background C3 deposition obtained with either heat-treated serum or serum containing 10 mM EDTA was comparable with that of VBS only controls.

Expression of rat C inhibitors by RAd infection protects human fibroblasts from rat C-mediated lysis

Over a range of MOIs, each of the adenoviruses conferred protection on human fibroblasts from rat C lysis (Fig. 5). RAdDAF and RAdCD59 at MOIs of 100 and 300 conferred significant protection on infected cells when compared with control virus (p < 0.001 for each comparison). RAdCrry, although less effective, also offered a significant degree of protection over control virus (p < 0.05 and p < 0.01 for MOI of 100 and 300). RAdCD59 and RAdDAF gave significantly better protection than RAdCrry (p < 0.01 for RAdCD59 vs RAdCrry and p < 0.001 for RAdDAF vs RAdCrry at a MOI of 100). There was no significant difference between RAdDAF and RAdCD59 at any of the MOIs tested. RAd35, at an MOI of 30, conferred a small but significant degree of protection from lysis over uninfected controls (p = 0.02).

Double infection of human fibroblasts with combinations of virus confers increased protection

We wished to examine whether pairs of regulators inhibiting C at the same or different stages expressed on the same cell could act synergistically to protect from lysis. Double infection of a HFFF cell population was confirmed by infecting cells singly with virus at a MOI of 200, or doubly, with each virus at a MOI of 200 and comparing expression levels by flow cytometry. At this MOI, all cells expressed and expression levels of each individual protein in dual-infected cell populations were equal to those obtained in single infected populations (data not shown). These data demonstrate dual infection of single cells with each adenovirus. Comparison of single infections with each virus at a MOI of 200 with double...
intraarticular injection of a RAd encoding b demonstrated the expression of b in relevant rat models of disease. Roessler et al. (37) have regulators in this system should permit sustained, local C inhibition in quiescent cells to a high level in vivo (36). Expression of the rat C for delivery because of its capacity to infect both replicating and nonreplicating cells over weeks or months (33–35). We have chosen RAd as a vehicle to overcome the problem of immunogenicity in models, enabling the testing in vivo of long term therapy with C regulators. A second problem is that the repeated systemic administration of C regulators might render the individual more susceptible to infection. It has also been recognized, in large part because of the successes obtained in animal models with agents such as soluble human C receptor 1. Animal studies to date have been limited to acute situations, because of the short half-life and immunogenic nature of human C regulators in rodents (12). To develop therapies for chronic conditions such as rheumatoid arthritis, C inhibition will need to be sustained over long periods in vivo. Using the rodent C regulators should enable the recognition of C inhibitors in therapy is now well documented, as the potential for utilizing C regulators in therapy is now well understood.

Discussion

The potential for utilizing C regulators in therapy is now well recognized, in large part because of the successes obtained in animal models with agents such as soluble human C receptor 1. Animal studies to date have been limited to acute situations, because of the short half-life and immunogenic nature of human C regulators in rodents (12). To develop therapies for chronic conditions such as rheumatoid arthritis, C inhibition will need to be sustained over long periods in vivo. Using the rodent C regulators should overcome the problem of immunogenicity in models, enabling the testing in vivo of long term therapy with C regulators. A second problem is that the repeated systemic administration of C-regulatory proteins will be technically difficult and prohibitively expensive. A possible solution to these problems is to utilize gene therapy to express multiple C regulators will further enhance protection of cells.

RAd delivery of β-gal to rat synovium via RAd35

Sections of synovium from rat knees injected with RAd35 showed abundant areas of blue stain, corresponding to infected cells (data not shown). Sections from the concomitant control knee were negative.

Table 1. Reduction in C3 deposition on human fibroblasts by expression of rat C inhibitors using recombinant adenovirus vectors

<table>
<thead>
<tr>
<th>MOI</th>
<th>RAdCD59 (MCF)</th>
<th>RAdCrry (MCF)</th>
<th>RAdDAF (MCF)</th>
<th>RAdCrry + RAdDAF (MCF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>37.96 ± 2.74</td>
<td>37.96 ± 2.74</td>
<td>37.96 ± 2.74</td>
<td>19.26 ± 3.04</td>
</tr>
<tr>
<td>100</td>
<td>29.95 ± 2.71</td>
<td>27.30 ± 1.67</td>
<td>23.69 ± 2.49</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>35.42 ± 6.11</td>
<td>24.01 ± 6.19</td>
<td>19.86 ± 2.48</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>35.73 ± 4.13</td>
<td>21.30 ± 1.23</td>
<td>18.40 ± 2.61</td>
<td></td>
</tr>
<tr>
<td>100 + 100</td>
<td>6.11</td>
<td>1.23</td>
<td>2.71</td>
<td>2.74</td>
</tr>
</tbody>
</table>

*Human fibroblasts were infected at MOIs of 100, 200, and 300 with RAdCD59, RAdCrry, or RAdDAF or left uninfected. A combination infection was also performed with RAdDAF and RAdCrry each at an MOI of 100. On day 3 p.i., cells were exposed to rat serum and C3 measured by flow cytometric analysis with mAb C3/30 as primary Ab. Secondary detection was with goat anti-mouse PE conjugate. Experiments were done in triplicate and results are shown as mean cell fluorescence (MCF) ± SD of triplicate measurements from a typical experiment.

generated an adenovirus recombinant encoding human CD59 and demonstrated expression of human CD59 on rat endothelial cells infected with the virus.

Here we describe the generation of adenovirus recombinants expressing rat CD59, rat DAF, and rat Crry. Infection of human fetal fibroblasts with each of these viruses caused high levels of membrane expression of the corresponding C regulator. Expression was dependent on the dose of adenovirus used. The expressed proteins were similar in size to the endogenous regulators on rat erythrocytes. The GPI-anchored proteins, CD59 and DAF, were efficiently cleaved from expressing cells by treatment with PIPLC, indicating that the anchor signal was correctly processed. Transmembrane Crry was not released by PIPLC but instead showed an apparent increase in expression, likely due to increased accessibility of the protein to Ab after PIPLC release of other membrane proteins.

Each of the expressed proteins conferred protection against attack by rat C. In C lysis assays, RAdCD59 and RAdDAF were equally effective, and each provided human fibroblasts with better protection than did RAdCrry. This was surprising, given the known importance of Crry in protection against C attack in vivo (39–41). This result may be attributable to the expression system in that RAdDAF and RAdCD59 might promote higher levels of expression of their transgene than RAdCrry. Indeed, at very high MOIs of RAdCrry, inhibition of lysis did reach the levels achieved with RAdCD59 and RAdDAF (data not shown). The differences may also relate to the lytic assay used, in which C activation was

FIGURE 5. Protection of HFFF from rat C-mediated lysis with RAd. Fibroblasts were infected in triplicate with RAdCD59, RAdCrry, RAdDAF, or RAd35 at MOIs of 100, 200, and 300. On day 3 p.i., the cells were Ab sensitized and exposed to rat C. Experiments were done in triplicate, and results are shown as mean ± SD of triplicate measurements from a typical experiment.
achieved through the classical pathway. It is possible that rat DAF functions better in the classical pathway whereas rat Crry favors the alternative pathway. Although this possibility has not been formally tested in rats, in humans, MCP, which has many features in common with Crry, functions only in the alternative pathway (42, 43). Infection with either RAdDAF or RAdCrry inhibited C3b deposition, the former again being more active in the assay used. RAdCD59, acting on the terminal pathway had no effect on C3b deposition, the results being more active in the assay used.

FIGURE 6. Effect of expression of combinations of rat C regulators. Fibroblasts were infected in triplicate either with a single virus, RAdCD59, RAdCrry, or RAdDAF each at a MOI of 400, or with a combination of two viruses each at an MOI of 200. At day 3 p.i., the cells were Ab sensitized and exposed to rat C. Experiments were done in triplicate, and results are shown as mean ± SD of triplicate measurements from a typical experiment.

scribed here thus provide useful tools for examining the effects of C inhibitors on these difficult cells. For example, we have preliminary evidence that these adenovirus vectors infect rodent brain and induce abundant expression of C regulators on neurons and glia. For use in therapy, either cells can be infected ex vivo and injected into the target site or the adenovirus can be injected directly into the target site to infect cells in situ. We have chosen initially to express the C regulators as membrane-anchored molecules. Only direct injection of virus will thus be effective in obtaining protection of cells in the target site. Adenovirus vectors encoding soluble forms of the C regulators offer an alternative approach to inhibiting C activation at a target site; these could be administered either directly or after cell infection ex vivo.

Our primary target for therapy with the adenovirus vectors is inflammatory joint disease. A role for C has been clearly demonstrated in human arthritis and in animal models (44–48). Both systemic and local therapy with C regulators has been shown to inhibit disease in several rodent models of arthritis (13, 49, 50). Adenovirus recombinants are particularly suited to therapy of arthritis because of the ease of access to the joint space. In a recent study, adenovirus vector encoding a modified form of the TNF-α receptor was delivered directly into the rat joint and was effective in suppressing collagen-induced arthritis (51). Our preliminary studies utilizing the RAd35 vector expressing β-gal demonstrate that sustained, high level expression of the gene can be attained in synovium by this approach without inducing inflammation. Studies of the efficacy of the C regulator-encoding adenoviruses administered intraarticularly in Ag arthritis are in progress.

References


