AP-1 and STAT-1 decoy oligodeoxynucleotides attenuate transplant vasculopathy in rat cardiac allografts

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Aims Cardiac allograft vasculopathy (CAV) continues to be an unsolved clinical problem requiring the development of new therapeutic strategies. We have previously demonstrated that ex vivo donor allograft treatment with decoy oligodeoxynucleotides (ODN) targetting the transcription factors, activator protein-1 (AP-1) or signal transducer and activator of transcription-1 (STAT-1), delays acute rejection and prolongs cardiac allograft survival. Here, we investigated whether this treatment regime also prevents the occurrence of CAV in a fully allogeneic rat heart transplantation model.

Methods and results Wistar-Furth rat cardiac allografts were perfused ex vivo with AP-1 decoy ODN, STAT-1 decoy ODN, or buffer solution and transplanted into the abdomen of Lewis rats immunosuppressed with cyclosporine. Treatment with both decoy ODNs but not vehicle significantly attenuated the incidence and severity of CAV. Laser-assisted microdissection/real-time polymerase chain reaction as well as immunohistochemistry analyses revealed a significant increase in CD40 abundance in the coronary endothelial cells and medial smooth muscle cells on day 1 post transplantation which was virtually abolished upon AP-1 or STAT-1 decoy ODN treatment. While the AP-1 decoy ODN primarily attenuated basal CD40 expression, the STAT-1 decoy ODN suppressed tumour necrosis factor-α/interferon-γ-stimulated expression of CD40 in rat native endothelial cells.

Conclusion Treating donor hearts with decoy ODNs neutralizing AP-1 or STAT-1 at the time of transplantation prevents upregulation of CD40 expression in the graft coronary arteries and effectively inhibits CAV.

KEYWORDS
Heart;
Transplantation;
Atherosclerosis;
Decoy oligodeoxynucleotide;
CD40

1. Introduction
Cardiac allograft vasculopathy (CAV) is an aggressive and diffuse form of atherosclerosis affecting large epicardial arteries and small penetrating intramyocardial branches in cardiac allografts.1 CAV emerges within months to years following transplantation and exhibits pathological features that are clearly distinct from classical atherosclerosis.2 Nonetheless, endothelial dysfunction plays a pivotal role also in CAV which is reflected by the severe impairment of endothelium-dependent vasodilatation early after transplantation.3 Contributing factors include pre-existing atherosclerosis of the graft vessels, ischaemia-reperfusion injury, brain death of the donor, denervation, disruption of the lymphatic system, acute and chronic immune injury, viral infection, exposure to immunosuppressive drugs as well as classical risk factors such as diabetes, dyslipidaemia, or hypertension.4 The dysfunctional allograft endothelial cells step up expression of adhesion molecules, chemokines, and cytokines, as well as co-stimulatory molecules for T and B cell activation. In turn, activated recipient T-helper cells stimulate the expression of class II major histocompatibility complex molecules on the endothelial cells, thus potentially evoking a sustained alloimmune reaction.2,4,5 With an estimated 10% incidence per year, CAV remains to be the leading cause of graft failure and death in long-term heart transplant recipients4 and continues to be an unsolved pressing clinical problem.2–8

The search for pharmacological therapeutic options in CAV has stimulated an intense effort for seeking out agents that might be beneficial in retarding or regressing its development. Statins and calcium-channel blockers were shown to have some limited protective effect.4 Although more effective immunosuppressive regimens comprising proliferation signal inhibitors such as sirolimus or everolimus might prove to be better in attenuating coronary artery
Decoy ODN attenuation of graft arteriosclerosis

2. Methods

2.1 Animals

Inbred male Lewis (LEW, RT11) and Wistar-Furth (WF, RT1u) strain rats were purchased from Harlan Winkelmann (Borchen, Germany) and housed in the animal care facility at the Department of Surgery, University of Giessen, Germany. The animals were kept under standard temperature, humidity, and timed light conditions and provided with rat chow and water ad libitum. Animals were treated in a humane manner in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). The transplant procedures were performed when animals attained a body weight of approximately 250 g. LEW rats served as recipients of WF cardiac allografts or LEW isografts, respectively.

2.2 Cardiac allograft model

A total of 38 WF hearts (allografts) and seven LEW hearts (isografts) were transplanted to the infrarenal great vessels of the recipient LEW rats by standard microvascular surgery in the modified technique of Ono and Lindsey,10 as previously described.11 Briefly, after the recipient and donor rat had been anesthetized by intramuscular injection of ketamine (10 mg/100 g body weight) and xylazine (1 mg/100 g body weight), the abdominal great vessels of the donor and recipients were carefully separated. The donor heart was harvested and flushed with Ringer solution (Na+ 147 mmol/L, K+ 4 mmol/L, Ca2+ 2 mmol/L, Cl− 156 mmol/L). Thereafter, the WF allografts were perfused with either 4 mL of AP-1 decoy ODN (5 µmol/L), STAT-1 decoy ODN (5 µmol/L), or vehicle [appropriate dilution of TEN buffer (10 mmol/L Tris, 1 mmol/L EDTA, and 150 mmol/L NaCl) in Ringer solution] and exposed to 45 min of warm ischaemia. During this time, the donor aorta was anastomosed to the recipient abdominal aorta with 7–0 prolene in an end-to-side technique. The donor pulmonary artery was anastomosed to the recipient inferior vena cava end-to-side. The trunks round the recipient’s great vessels were released after a total period of 45 min and reperfusion of the graft was started. Within seconds both types of grafts started beating. Thereafter, the abdomen was closed by sutures and the graft function was monitored daily by palpation rated on a scale of 0–3 (0 = no mechanical activity; 3 = vigorous normal beats). All the recipients were treated with cyclosporine A (Novartis, Basel, Switzerland) subcutaneously from the day of transplantation until sacrifice 100 days later. The dosage of 5 mg/kg body weight per day had been established to fully suppress acute rejection in a previous series of experiments with the same transplantation model. According to this study, CAV was not apparent after 30 days but fully established at 60 days post-transplantation and maintained on this level thereafter.

2.3 Decoy decoy oligodeoxynucleotides technique

Double-stranded ODNs (purity >95%) were prepared from complementary single-stranded phosphorothioate bonded ODNs obtained from Eurogentec (Cologne, Germany) as previously described.9 The resulting 400 µmol/L solution in TEN buffer was split into 50 µL aliquots and frozen at −80 °C until further use. They were defrosted and dissolved in Ringer solution (allograft perfusion) or Waymouth medium (incubation of rat aortic segments) yielding a final concentration of 5 or 10 µmol/L, respectively. The sequences of the single-stranded ODNs were as follows (bold letters denote phosphorothioate-bonded bases, mutations are set in italics):

AP-1 decoy ODN: 5'-CGTTTATGTACTGACGGCGAA-3'
AP-1 mutated control ODN: 5'-CGTTTATGTACTTAGCCGGAA-3'
STAT-1 decoy ODN: 5'-CATTTATGCATATCCTCTGAAGTG-3'
STAT-1 scrambled control ODN: 5'-TGTCATACTCGTACACAC-3'

2.4 Histology

The cardiac allografts and the native hearts of the recipients were removed 100 days post transplantation. A total of nine native non-transplanted donor hearts served as controls. The explanted hearts were serially sectioned into 2–3 mm slices, embedded in Tissue-Tek optimal cutting temperature (OCT) compound, snap-frozen in liquid nitrogen precooled isopentane, and stored at −80 °C. After fixation in ice-cold acetone for 10 min, cryostat sections (5 µm) were dried and stained with haematoxylin & eosin (H&E) and elastic van Gieson. They were examined by standard light microscopy and scored for both the grade of rejection, using a scale of 0 to 2 (0 = no rejection; 2 = severe rejection with massive mononuclear cell infiltrate), and the degree of graft atherosclerosis/intimal thickening using a scale of 0 to 5 (Figure 1) as described by Adams et al.12 Only vessels that were cut orthogonally and displayed a clear internal elastic lamina staining were scored. Histopathological analysis was performed by an experienced pathologist (R.M.B.) independently and blinded from the treatment assignment of each animal.

2.5 Laser-assisted microdissection and isolation of total RNA

In a separate series of experiments, cryostat sections were prepared from cardiac allografts 1 day post transplantation and native non-transplanted donor hearts as described above (three hearts in each group). For microdissection, two cryostat sections of each heart were mounted on PALM slides (PALM membrane slides, P.A.L.M. Microlaser Technologies, Bernried, Germany). The slides were pretreated by UV illumination (UV wavelength 254 nm) for 30 min using a UV lamp (MinUVIS, Sarstedt, Nürnberg, Germany). Later, the slides were stained with Hemalaun for 1 min, then consecutively immersed in tap water, 70% ethanol, 96% ethanol, and 100% ethanol, and subsequently air-dried. Cell samples comprising intimal endothelial cells and media cells (25–30 cell profiles each) were collected from the coronary arteries by laser microdissection using the PALM laser-pressure catapulting device (PALM MicroBeam) together with the PALM Robo Software (P.A.L.M. Microlaser Technologies). The microdissected cells were catapulted into the cap of a 1.5 mL reaction tube containing 2 µL mineral oil (PCR grade, Sigma-Aldrich, Deisenhofen, Germany). Later, 350 µL
homogenization buffer and 3.5 μL β-mercaptoethanol were added, followed by inverse gentle shaking, centrifugation, immediate freezing in liquid nitrogen, and storing at -80°C. Total RNA was isolated from the picked cells using the RNEASY® micro kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

2.6 Real-time polymerase chain reaction analysis

Relative mRNA quantification with porphobilinogen deaminase (PBGD) as housekeeping gene was performed by real-time polymerase chain reaction (PCR) using the Sequence Detection System 7700 (PE Applied Biosystems, Foster City, CA, USA) essentially as described previously. Amplification efficiency for PBGD was calculated by serial dilutions of the PCR product and the slope of the resulting standard curve to 0.90 ± 0.02 and thus approximately equal to that of CD40. Thereafter, each sample of microdissected cells was divided, for the analysis of CD40 as target gene and PBGD as housekeeping gene, into two aliquots of 10 μL. The TaqMan Universal Master Mix (PE Applied Biosystems) was used according to the manufacturer’s instructions. Ten microlitres of cDNA and oligonucleotide primers (CD40: sense, 5'-GAAGACCACAAATGCACGCCACGCACGCA-3' and antisense, 5'-GCCCTTGATCGTGCGAG-3'; PBGD: sense, 5'-ATGGCCCGTGAAAGGCAGGCG-3' and antisense, 5'-CAGCATGCCACCGTCTCTCTCC-3') were added at a final concentration of 300 nmol/L each, and hybridization probes (CD40: 5'-TCAGGCGAATTCTCAGCTCACTGGAACA-3'; PBGD: 5'-CCACCTGACACAGCTGAGACACAGAGAC-3') at a final concentration of 200 nmol/L in a total volume of 50 μL. Cycling conditions were adapted to 95°C for 10 min, followed by 45 cycles of 95°C for 15 s, and 60°C for 1 min. Primers and probes were purchased from metabion (Martinsried, Germany).

2.7 Immunohistochemistry

In a separate series of experiments, cryostat sections were prepared from cardiac allografts 1 day post transplantation (five hearts in each group) and a total of three native non-transplanted donor hearts as described above. Antigen retrieval was performed using the microwave-citrate salt method (10 mmol/L sodium citrate pH 6.0, microwave oven set to 600 W for 15 min). After blocking endogenous peroxidase activity and incubation with normal goat serum, the sections were stained with a rabbit anti-human CD40 antibody (dilution 1:1000, Santa Cruz Biotechnology, Heidelberg, Germany) in combination with a 3,3'-diaminobenzidine-based enhanced detection method (Envision®TM) according to the manufacturer’s (DAKO, Glostrup, Denmark) instructions. After H&E counterstaining, the sections were mounted with Aquamount (Gurr, Hanau, Germany). Positive immunoreactivity was documented using a SPOT RT colour charged-coupled device camera (Diagnostic Instruments, Sterling Heights, MI, USA) coupled to an Axiolovar S100 TV microscope (Zeiss, Jena, Germany) and the MetaMorph 6 software package (Universal Imaging, West Chester, PA, USA).

Positive immunoreactivity was examined by standard light microscopy first by counting the number of CD40-positive blood vessels in a total of 34 ± 2 blood vessels looked over per section (n = 18) at 100- to 200-fold magnification. In addition, CD40 immunoreactivity in the endothelial and medial smooth muscle cell layer (of the CD40-positive blood vessels) was scored at 200- to 400-fold magnification using a scale of 0 to 4 (0, absent; 1, low; 2, moderate; 3, strong; 4, very strong staining). Finally, the number of CD40-positive leukocytes, primarily located in the tissue surrounding the blood vessels, was scored at 100- and 200-fold magnification in these sections using a scale of 0 to 4 (0, absent; 1, low; 2, moderate; 3, strong; 4, severe). The aforementioned evaluations were

![Figure 1](image-url) Representative photomicrographs of rat cardiac allografts illustrating coronary artery histology. (A) Grade 0: Normal coronary artery. (B) Grade 1: Intimal thickening ≤ 50% of the perimeter of the lumen with ≤ 20% stenosis. (C) Grade 2: Intimal thickening involving 50–100% of the perimeter of the lumen with < 20% stenosis. (D) Grade 3: Circumferential intimal thickening with 20–50% stenosis. (E) Grade 4: Circumferential intimal thickening with 50–80% stenosis. (F) Grade 5: Circumferential intimal thickening with > 80% stenosis. Original magnifications ×100; haematoxylin&eosin/elastic van Gieson stain.
2.8 Preparation and incubation of ring segments of rat thoracic aorta

Aortas (1.5 mm internal diameter) were isolated from pentobarbitone-anaesthetized male Wistar rats (200–250 g body weight) as described previously\(^\text{14}\) and cut into 5–7 mm long segments which were placed in 1 mL Waymouth medium containing 10% foetal bovine serum. The segments were incubated with either the AP-1 decoy ODN or a mutated control ODN (10 \(\mu\)mol/L each) for 8 h to monitor their effects on basal CD40 expression. In another set of experiments, they were incubated for 16 h in the absence or presence of rat interferon-\(\gamma\) (IFN-\(\gamma\), 200 U/mL) plus tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\), 500 U/mL) with or without pre-treatment (4 h) with either the STAT-1 decoy ODN or a scrambled control ODN (10 \(\mu\)mol/L each). To verify that CD40 expression is primarily detected in the endothelium, one segment was routinely denuded prior to incubation in the medium.

2.9 Western blot analysis

Homogenates of the rat aortic segments were prepared as described previously\(^\text{14}\). The resulting protein extracts were separated by denaturing 10% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate according to standard protocols and then transferred to a BioTrace polyvinylidene fluoride transfer membrane (Pall Corporation, Rosdorf, Germany). For detection of CD40, the immobilized proteins were consecutively exposed to a polyclonal rabbit anti-CD40 antibody (1:2500 dilution, Calbiochem/Merck Biosciences, Darmstadt, Germany), an anti-rabbit immunoglobulin (IgG) horseradish peroxidase (HRP) conjugate (1:5000 dilution; Sigma–Aldrich), and the ECL Plus Western Blotting Detection system (Amersham/GE Healthcare, Munich, Germany). For visualization, documentation and quantization of the detected bands, the ChemiDoc XRS System and the Quantity One 1-D analysis software (Bio-Rad Laboratories, Munich, Germany) were used. Loading and transfer of equal amounts of protein in each lane was verified by reprobing the membrane with a monoclonal anti-\(\beta\)-actin antibody from mouse ascitis fluid and an anti-mouse IgG-HRP conjugate (both antibodies obtained from Sigma–Aldrich, 1:3000 dilution).

2.10 Statistical analysis

Unless indicated otherwise, results are expressed as means \(\pm\) SEM. Statistically significant differences between experimental and control groups were calculated using the Kruskal–Wallis test followed by Dunn’s post-test (pair-wise comparisons of all groups) for the histopathology data, two-tailed Fisher’s exact test for the real-time PCR data, or one-way analysis of variance followed by the Bonferroni test for selected pairs of groups for the immunohistochemistry and Western blot data. The utilized statistics software was Instat version 3.00 (GraphPad, San Diego, CA, USA) with \(P < 0.05\) considered significant.

3. Results

3.1 General observations

Cardiac allografts with either decoy ODN or vehicle treatment all survived in the recipients for the 100 days observation period, and there was no difference with respect to the graft function score (control group: 2.8 \(\pm\) 0.2; AP-1 dODN group: 3.0 \(\pm\) 0.0; STAT-1 dODN group: 2.9 \(\pm\) 0.1; not significant). Decoy ODN treatment did not affect general behaviour or body mass development.

3.2 Histopathological findings

At the time of sacrifice (100 days post transplantation), neither the transplanted allografts (\(n = 14\)) nor the isografts (\(n = 7\)) displayed significant signs of myocardial rejection (rejection score < 1). On the other hand, the allografts but not the isografts had developed coronary vascular...
lesions indistinguishable in appearance from human accelerated graft vasculopathy. Early lesions demonstrated patchy endothelial protrusions, endothelial swelling, and adherence of a few mononuclear cells along the vessel intima. More advanced lesions were characterized by a marked cellular expansion of the intima and an inflammatory infiltrate typically found within the internal elastic lamina and in a halo zone exterior to the adventitia. Occasionally, the internal elastic membrane was stretched and focally disrupted in advanced lesions. Diffuse fibrointimal thickening that markedly compromised the lumen resulted in a virtually complete occlusion of some coronary arteries. Representative photographs of coronary arteries showing progressive allograft vasculopathy (CAV) in the stages as defined by Adams et al.12 are depicted in Figure 1.

A total of 538 coronary artery cross-sections were analysed for the development of transplant vasculopathy in this study. In the control group (TEN buffer), 76.2% of the coronary vessels were affected by transplant vasculopathy. In contrast, both the AP-1 and the STAT-1 decoy ODN-treated allografts revealed a significantly lower frequency of the disease with 43.4% and 30.6% affected vessels, respectively (Figure 2A). In addition, the severity of transplant vasculopathy, scored on a scale of 0 to 5, was significantly lower in the decoy ODN-treated allografts when compared with the respective control group (Figure 2B). Furthermore, in both the AP-1 and STAT-1 decoy ODN group, the distribution of vessels was shifted towards less severe luminal occlusion (Figure 2C).

3.3 CD40 expression in transplant coronary endothelial and media cells

As shown previously,9 perfusion of the donor hearts with Texas Red-labelled AP-1 or STAT-1 decoy ODN resulted in an almost exclusive uptake of the double-stranded nucleic acids by the endothelial cells of the intima with some fluorescence detectable in the media but not in the adventitia or within the myocardial tissue. To quantify the effects of the two decoy ODNs in the expression of CD40, one of the primary target genes of STAT-1,15 laser microdissection followed by real-time PCR analysis of coronary endothelial and media cell mRNA was employed. These analyses revealed a significant upregulation of CD40 gene transcript levels in the control allografts at day 1 post transplantation, which was strongly attenuated (AP-1 decoy ODN) or nearly abolished (STAT-1 decoy ODN) upon treatment of the allografts with the decoy ODNs (Figure 3).

In a separate series of experiments, immunohistochemistry analysis confirmed these changes in CD40 mRNA abundance on the protein level. About 48-64% of the blood vessels analysed per section stained positive for CD40. Although there was a trend towards an increased percentage of CD40-positive blood vessels in the non-treated allografts when compared with the decoy ODN-treated allografts or non-transplanted control hearts, this was not statistically significant (Figure 4A). CD40 immunoreactivity in the coronary artery endothelial cells and medial smooth muscle cells of the non-transplanted control hearts was at the limit of detection (Figure 4D), but clearly enhanced in the non-treated control allografts at day 1 post transplantation (Figure 4B and D). Pre-treatment with the decoy ODNs significantly reduced (by 71%, STAT-1 decoy ODN) or abolished (AP-1 decoy ODN) this increase in CD40 immunoreactivity (Figure 4B and D). In addition, the number of CD40-positive leukocytes surrounding the vessel wall, which were absent in the non-transplanted control hearts, was significantly reduced in the decoy ODN-treated allografts (by 48%, STAT-1 decoy ODN; by 69%, AP-1 decoy ODN; Figure 4C).

3.4 Decoy ODN effects on CD40 expression in native rat endothelial cells

To verify that both decoy ODNs affect CD40 expression in rat endothelial cells, endothelium-intact segments of rat aorta were employed instead of rat cultured endothelial cells, which are difficult to isolate in sufficient quantity and purity. In these segments, CD40 expression mainly occurs in the endothelial cell layer as denudation resulted in a distinct loss of the protein (Figure 5). While basal CD40 expression was not affected by the STAT-1 dODN (Figure 5B), this was significantly attenuated upon administration of the AP-1 decoy ODN but not the corresponding scrambled control ODN (Figure 5A). The STAT-1 dODN but not the corresponding scrambled control ODN, on the other hand, completely abolished the TNF-α- plus IFN-γ-mediated rise in CD40 protein (Figure 5B).

4. Discussion

Allograft vasculopathy remains to be the leading cause of late cardiac allograft failure.2-8 It occurs in 50% or more of heart-transplant recipients in the first 5 years after transplantation. Current immunosuppressants taken by transplant recipients do not prevent the disease.6,8 Because of the long-standing failure of systemic therapy in the
prevention or retardation of CAV, gene therapeutic strategies have been used recently to regulate genes believed to be important in this process. Antisense ODNs against several inflammatory mediators have been employed with varying success in cardiac transplant models. Another approach to therapeutically regulate inflammatory mediators is to directly target the intracellular signalling pathways that transmit extracellular signals from membrane-expressed receptors downstream to the nucleus, resulting in transcriptional activation of specific genes. We have chosen AP-1 and STAT-1 as targets to impede the development of CAV not only because both transcription factors play a role in the cellular immune response, but also because we were able to demonstrate recently that decoy ODNs neutralizing these transcription factors delay acute rejection and prolong cardiac allograft survival in the very same rat heart transplantation model. To our knowledge this is the first report to show that ex vivo treatment with such nucleic acid-based drugs effectively protects against the development of arteriosclerosis in rat cardiac allografts. The retardation of transplant coronary artery disease observed with either decoy ODN in the present study most probably is because of inhibition of the expression of certain pro-inflammatory endothelial cell gene products downstream of transactivation of the respective transcription factor. One likely candidate is CD40, a major co-stimulatory molecule essentially involved in T-cell activation. CD40 is a member of the tumour necrosis factor receptor superfamily which is expressed on B cells, macrophages, dendritic cells, and endothelial cells as the counter receptor of CD154 found on activated T-helper cells and a fraction of cytotoxic T cells as well as other non-immune cells, e.g. activated platelets. CD40–CD154 interaction, resulting in the reciprocal activation of CD40-expressing cells via CD40 and the activation of T cells via CD154, is known to be crucial for the initiation and maintenance of the cellular as well as the humoral immune response. The importance of this co-stimulation for priming, expansion, and differentiation of T-helper cells, which in turn are believed to orchestrate the cytokine and growth factor-mediated intima proliferation in cardiac allograft vessels, has been convincingly revealed by earlier investigations.

In the present study, we found that CD40 expression is clearly upregulated both on the mRNA and protein level in coronary artery endothelial cells and to some extent also in the medial smooth muscle cells of rat cardiac allografts. These data confirm previous observations obtained in human and animal heart-transplant recipients. As demonstrated both by quantitative CD40 mRNA analysis of intimal endothelial and medial smooth muscle cells microdissected from the grafts and immunohistochemistry analysis of whole cryostat sections at day 1 after surgery, the two decoy ODNs similarly repressed this increase in CD40 expression most notably in the graft endothelium. These findings were supported by separate experiments with rat native endothelial cells in which the target transcription factors were shown to govern basal (AP-1) and cytokine-inducible CD40 expression (STAT-1), respectively. A specific effect of the two decoy ODNs on CAV is suggested by (i) the lack of effect of the corresponding mutated control ODNs on acute rejection in the same transplantation model, and (ii) the finding that both decoy ODNs, but not the corresponding mutated or scrambled control ODNs,
differentially affected CD40 protein levels in endothelium-intact segments of the rat aorta.

Given the central role of CD40–CD154 interactions in the development of acute and chronic allograft rejection, suppression of CD40, especially on the graft endothelium suggests a plausible mechanism by which either AP-1 or STAT-1 decoy ODN treatment effectively attenuates transplant arteriosclerosis. This interpretation is also in line with previous studies demonstrating that blockade of CD154 by the administration of a single dose of monoclonal anti-CD154 antibodies at the time of transplantation induces long-term survival of cardiac allografts in rodents as well as in primate recipients. It also confirms the clinical and experimental observation that immunological stimuli in the early phase after surgery are one of the major factors in the development of transplant arteriosclerosis. However, we cannot and explicitly do not rule out the possibility that in addition to CD40, inhibition of expression of other pro-inflammatory target genes in the graft endothelium and possibly also in the medial smooth muscle cells such as, e.g., ICAM-1 and VCAM-1, has contributed to the therapeutic effect of the decoy ODNs in our rat heart transplantation model. This may also be inferred from the findings that sole perioperative blockade of CD40–CD154 interactions is not sufficient to fully prevent CAV.

Decoy ODNs specifically neutralize their target transcription factor, thus blocking the expression of genes which are controlled by the said transcription factor; they do not normally block its expression. Their distinct advantage is that they are rapidly transported into their target cells without any additives and immediately thereafter can act on their target transcription factor. Both AP-1 and STAT-1 control the expression of many pro-inflammatory genes, so that neutralizing either transcription factors will result, as discussed above, in diminishing the expression of several target genes simultaneously. These nucleic acid-based drugs may thus be perceived as rather non-specifically acting when compared with the single target gene knockdown that is typically attained when employing an antisense ODN or siRNA approach. This relative lack of specificity, on the other hand, may explain why the STAT-1 decoy ODN, for example, exerts such a powerful glucocorticoid-like anti-inflammatory effect in various animal models of inflammation without any appreciable side-effects. Although specific for their target gene product, the other nucleic acid-based drugs face the problems of most likely being less efficacious, comparatively slow in onset depending on the turnover of their target protein and rather difficult to deliver into their target cells.

In conclusion, single application of a decoy ODN neutralizing either AP-1 or, in particular, STAT-1 during transplantation effectively inhibits CAV, mediated at least in part through the attenuation of CD40-mediated alloimmunogenicity of the graft endothelium in the first days following transplantation. The local use of ex vivo STAT-1 or AP-1 decoy ODNs might provide an attractive, feasible, highly effective therapeutic approach for retarding lesion formation in this particular coronary compartment.

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