Available online at www.sciencedirect.com
SCIENCE DIRECT®
ACADEMIC PRESS
Biochemical and Biophysical Research Communications 298 (2002) 10–16
BBRC
www.academicpress.com

# A double-strand decoy DNA oligomer for NF-κB inhibits TNFα-induced ICAM-1 expression in sinusoidal endothelial cells

Tomoyoshi Shibuya,[a] Yoshiyuki Takei,[a] Miyoko Hirose,[a] Kenichi Ikejima,[a] Nobuyuki Enomoto,[a] Atsushi Maruyama,[b] and Nobuhiro Sato[a,*]

[a] *Department of Gastroenterology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan*
[b] *Department of Biomolecular Engineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, Kanagawa 226-8501, Japan*

Received 13 September 2002

**Abstract**

Altered gene expression of liver sinusoidal endothelial cells (SECs) is associated with impaired immune response. Here we report that the decoy technique effectively suppresses TNFα-induced ICAM-1 expression in SEC. An NF-κB decoy (NF-κB31: 5′-TGGGGACTTTCCAGTTTCTGGAAAGTCCCCA-3), which contains a consensus sequence for NF-κB, was complexed to PLL-g-HA [hyaluronate-grafted poly(L-lysine) copolymer] that permits transfer of exogenous DNA selectively to the SEC. The PLL-g-HA/NF-κB31 complex was added to the culture media of LSE cells, a human SEC-derived cell line. Then, cells were stimulated with TNFα (5 ng/mL). PLL-g-HA/NF-κB31, but not control oligodeoxynucleotides having a reverse or scrambled sequence, inhibited the intranuclear localization of NF-κB induced by TNFα, with almost complete inhibition at 2.5 μg/mL as DNA. NF-κB31 attenuated the increase in ICAM-1 mRNA as well as protein levels in LSE cells. The decoy technique in combination with PLL-g-HA may provide a novel strategy for manipulation of SEC functions.


*Keywords:* Decoy; Sinusoidal endothelial cells; Nuclear factor-κB; Tumor necrosis factor-α; Intercellular adhesion molecule-1; Hyaluronate-grafted poly(L-lysine) copolymer

Liver sinusoidal endothelial cells (SECs) are strategically located at the interface between blood and parenchymal cells and play a pivotal role in modulating immunity and inflammation [1,2], cell death, and disturbance of microcirculation. Altered immunoresponses of the SEC are closely related to pathogenesis of viral hepatitis, fulminant hepatic failure, and transplant rejection [3]. In addition, we have shown that SECs are involved in liver fibrogenesis by expressing a functional leptin receptor Ob-Rb, through which leptin elicits intracellular signaling, leading to enhanced TGFβ expression [4].

Further, it has been proposed that "activation of the SEC" underlies the vascular aspects of liver pathophysiology such as systemic inflammatory response syndrome (SIRS) and xenotransplant rejection [5,6]. Activated SECs induce inflammation, phenomenon characterized by rapid gene expression in the SECs of proinflammatory mediators [7] and overexpression of adhesion molecules via the nuclear factor-κB (NF-κB) pathway [8].

NF-κB is a critical regulator of cytokine-inducible gene expression [9]. Proinflammatory cytokines, such as tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1), induce rapid nuclear translocation of NF-κB through degradation of inhibitor κB (IκB), an inhibitory cytoplasmic retention protein [9,10]. Genes regulated by NF-κB include chemokines involved in inflammatory responses, such as intercellular adhesion molecule-1 (ICAM-1) [9]. Thus, NF-κB is a key mediator of TNFα responses and an attractive target for therapeutic intervention to inflammation and inflammatory diseases.

We focused on the decoy technique [11–14] to block intracellular signaling pathways using double-strand

* Corresponding author. Fax: +81-3-3813-8862.
*E-mail address:* nsato@med.juntendo.ac.jp (N. Sato).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02369-0

oligodeoxynucleotide (ODN) directed at a cognate sequence of transcription factors of interest. We already reported a non-viral gene delivery system [hyaluronate-grafted poly(L-lysine) copolymer, PLL-g-HA] that permits targeted transfer of exogenous genes and ODNs selectively to the liver SEC in vivo [15,16]. Here we report that the decoy technique effectively suppresses TNFα-induced ICAM-1 expression in SECs.

## Materials and methods

*Cell culture.* Human liver sinusoidal endothelial cells (LSE) were obtained from Cell Systems (Kirkland, WA). LSE cells were established from normal human liver tissue by elutriation following dispase treatment of tissue. The LSE cells preserve the characteristics of SEC since they take up acetylated LDL, an endothelial-cell-specific substrate. LSE cells were cultured on type I collagen-coated polystyrene dishes using CS-C complete medium (Cell Systems) containing 10% FCS. Cells were incubated at 37 °C in a humidified atmosphere of 95% air–5% $CO_2$.

*Decoy ODNs.* An NF-κB decoy ODN, which contains a consensus sequence for NF-κB (NF-κB31: 5′-TGGGGACTTTCCAG TTTCTGGAAAGTCCCCA-3′) [17,18], was synthesized. This forms a double-strand DNA oligomer, with one end hinged by a stretch of T (Fig. 1A). Preliminary study showed that this "hairpin" structure confers resistance to exonucleases [19]. ODNs of similar structure containing a reversed sequence (Reverse ODN) or a scrambled sequence (Scrambled ODN) were synthesized for control experiments (Fig. 1A).

*Formulation of PLL-g-HA/ODN complex.* A gene carrier system [hyaluronate-grafted poly(L-lysine) copolymer, PLL-g-HA] was prepared by coupling HA to PLL in a 1:1 weight ratio using $NaBH_3CN_8$ as previously reported [15]. NF-κB31 and control ODNs were mixed with PLL-g-HA in PBS containing 8.1 mmol/L $Na_2HPO_4$, 2.68 mmol/L KCl, and 1.47 mmol/L $KH_2PO_4$, and 1 mol/L NaCl at a copolymer/DNA charge ratio ($[\text{amino group}]_{\text{copolymer}}/[\text{phosphate group}]_{\text{DNA}}$) of 2.0 and allowed to stand overnight at 4 °C before use.

*Uptake of Texas red-labeled PLL-g-HA/NF-κB in LSE cells.* The 5′ end of the NF-κB31 was labeled with Texas red. LSE cells were cultured in media containing the PLL-g-HA/Texas red-labeled NF-κB31 complex at a DNA concentration of 0, 1.25, 2.5, or 5 μg/mL. Subsequently, cells were fixed with ice-cold ethanol and rinsed with PBS. The coverslips were mounted in 80% glycerol in PBS. Cells were observed under a fluorescence microscope Axioplane (Carl Zeiss Japan, Tokyo, Japan).

*Electrophoretic mobility shift assay (EMSA).* Nuclear extracts were prepared from LSE cells treated with or without TNFα (20 ng/mL) for 1 h, according to the method of Dignam with slight modifications [20]. Binding reactions were performed for 20 min on ice in a buffer containing 10 μg nuclear extracts, 5 μg poly[d(I–C)], 10 mM Hepes, pH 7.6, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol, 5 mM $MgCl_2$, and 20,000 cpm $^{32}$P-labeled ODN containing consensus sequence of NF-κB element (5′-AGTTGAGGGGACTTTCCCAGGC-3′, Santa Cruz Biotechnology, Santa Cruz, CA). For competition experiments, unlabeled ODN containing consensus sequence of NF-κB element (Santa Cruz), unlabeled NF-κB31 or unlabeled Scrambled ODN, all in 250-fold excess, were added in the binding reactions and co-incubated. DNA–protein complexes were separated by non-denaturing polyacrylamide gel (5% in 0.4× TBE) electrophoresis. Gels were dried and exposed to X-OMAT films (Kodak, Rochester, NY).

*Reverse transcription-PCR.* PLL-g-HA/ODN (2.5 μg/mL as DNA) was added to the culture media of LSE and the cells were cultured for additional 3 h. Subsequently, the cells were further cultured in new media containing TNFα (5 ng/mL). After a culture period of 2 h, total RNA was extracted from LSE cells and expression of ICAM-1 mRNA was determined by RT-PCR [4]. PCR primers for ICAM-1 mRNA were as follows: sense 5′-CATAGAGACCCCGTTGCCTA-3′, antisense 5′-CTGACAAGTTGTGGGGGAGT-3′.

*Immunostaining of NF-κB and ICAM-1.* LSE cells were cultured in media containing PLL-g-HA/ODN (2.5 μg/mL as DNA) for 3 h before the addition of TNFα (5 ng/mL). Cells were fixed with ice-cold ethanol 1–4 h after addition of TNFα. After blocking with 1% goat serum in PBS for 30 min, cells were stained with an anti-p65 NF-κB antibody (Santa Cruz Biotechnology) or an anti-ICAM-1 (CD54) antibody (BD Biosciences, San Jose, CA) for overnight at 4 °C. An anti-rabbit or an anti-mouse goat IgG conjugated with FITC (Santa Cruz Biotechnology) was used as a secondary antibody. Subsequently, the relative fluorescence of cytoplasm and a nucleus was quantified on Photoshop (Adobe, San Jose, CA) program and the ratio was calculated.

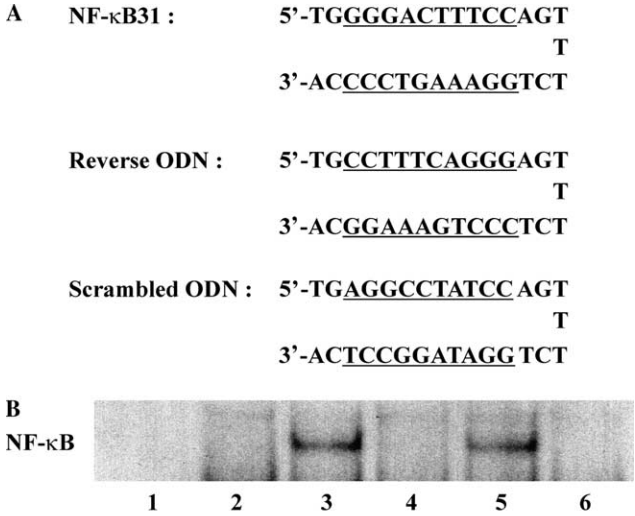


Fig. 1. Structure of hairpin DNA oligomers (A) and their binding ability to NF-κB (B). (A) An NF-κB decoy oligodeoxynucleotide (ODN) (NF-κB31: 5′-TGGGGACTTTCCAGTTTCTGGAAAGTCCCCA-3′), which contains a consensus sequence for NF-κB, was synthesized. ODNs of the identical structure having a reversed sequence of NF-κB (Reverse ODN) or a scrambled (random) sequence (Scrambled ODN) were used for control experiments. (B) Nuclear extracts were prepared as described in Materials and methods. Lane 1, incubation with probe alone (negative control); lane 2, nuclear extract from LSE cells that had not been stimulated with TNFα, incubated with $^{32}$P-labeled ODN containing consensus sequence of NF-κB element; lane 3, nuclear extract from LSE cells that had been stimulated with TNFα (20 ng/mL), incubated with $^{32}$P-labeled ODN (positive control); lane 4, nuclear extract from LSE cells that had been stimulated with TNFα (20 ng/mL) incubated with 250-fold excess of $^{32}$P-unlabeled ODN containing consensus sequence of NF-κB element; lane 5, nuclear extract from LSE cells that had been stimulated with TNFα (20 ng/mL) incubated with 250-fold excess of unlabeled Scrambled ODN; lane 6, nuclear extract from LSE cells that had been stimulated with TNFα (20 ng/mL) incubated with 250-fold excess of unlabeled NF-κB31.

## Results

### *Binding ability of the NF-κB31 decoy to NF-κB*

First, we confirmed that the NF-κB31 can bind NF-κB. This was performed by EMSA in which NF-κB31 was tested for its ability to compete with a $^{32}$P-labeled ODN containing an NF-κB consensus sequence. As shown in Fig. 1B, lane 3, TNFα (20 μg/mL) increased NF-κB DNA binding ability in LSE cells. Two hundred and fifty-fold excess of unlabeled NF-κB31 (lane 6), as well as consensus ODNs (lane 4), completely abolished the NF-κB binding ability. On the contrary, Scrambled ODN (lane 5) failed to inhibit the binding. These indicate that the NF-κB31 can bind to a consensus sequence for NF-κB.

### *Effect of PLL-g-HA on uptake of NF-κB31 by LSE cells*

After NF-κB31 labeled with Texas red was added to the culture media, cells were observed chronologically under a fluorescence microscopy. Intracellular fluorescence derived from Texas red was barely detectable for the incubation period up to 24 h (Fig. 2). In marked contrast, numerous bright spots in the cell, particularly in cytoplasm around cell nuclei, were clearly seen at 3 h when NF-κB31 was included in the culture media in a PLL-g-HA-complexed form, and the fluorescence intensity increased in a dose- (Fig. 2) and time-dependent manner (data not shown).

### *Effect of NF-κB31 on TNFα-induced translocation of NF-κB into cell nuclei*

We then examined if translocation of NF-κB protein into the nuclei of LSE cells induced by TNFα could be affected by the PLL-g-HA/ODN complexes. Stimulation of LSE cells with 5 ng/mL TNFα resulted in a dramatic accumulation of NF-κB protein in the cell nuclei as compared to the control cells in which no TNFα was added (Fig. 3A). The ratio of fluorescence intensity in cell nuclei versus cytosol increased from $0.85 \pm 0.04$ to $2.04 \pm 0.13$ (Fig. 3B, $p < 0.001$). In marked contrast, LSE cells that had been cultured for 3 h in media containing PLL-g-HA/NF-κB31 (2.5 μg/mL as DNA) prior to TNFα addition, the nuclear localization of NF-κB was abrogated almost completely (Figs. 3A and B, $*p < 0.01$). On the contrary, control ODNs having reverse or scrambled sequences did not inhibit the TNFα-induced nuclear translocation of NF-κB protein (Figs. 3A and B, # ns).

### *Effect of PLL-g-HA/NF-κB31 on TNFα-induced upregulation of ICAM-1 mRNA and protein*

TNFα stimulates the expression of ICAM-1 via a pathway dependent on the transcription factor NF-κB. Hence, we determined the effect of NF-κB31 on the amounts of ICAM-1 mRNA in LSE cells after stimulation with TNFα. The ICAM-1 mRNA was increased in LSE cells profoundly when stimulated for 2 h with

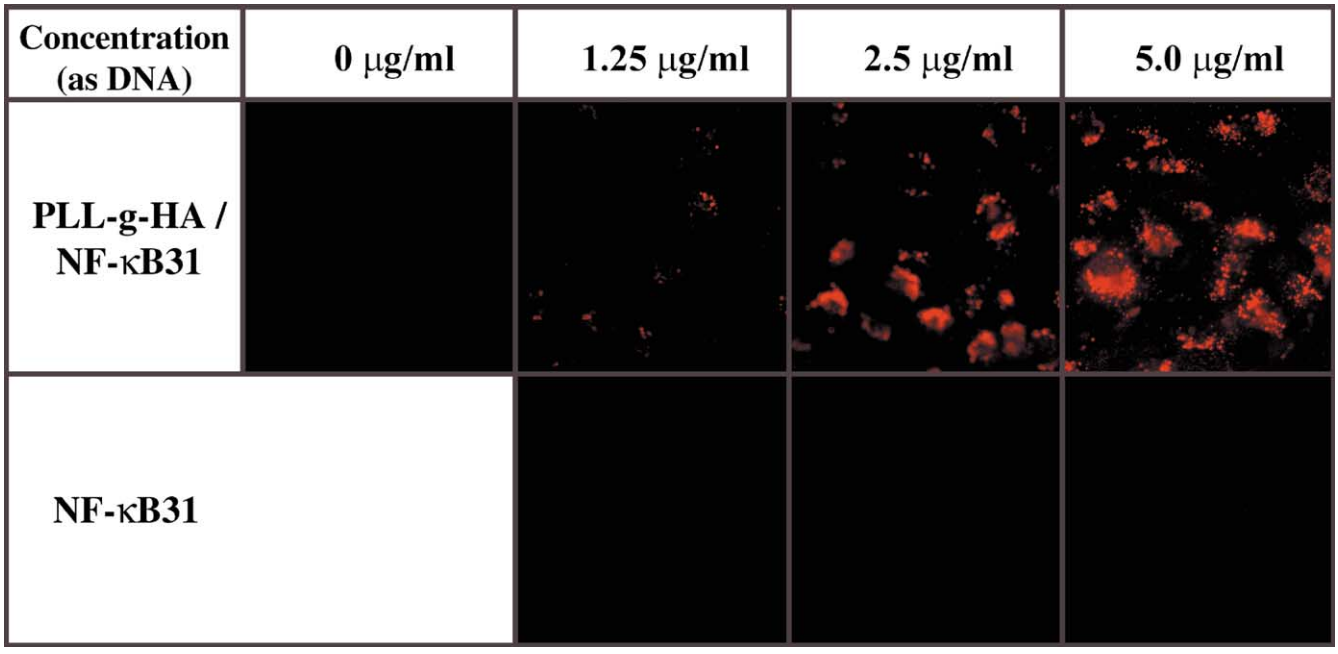


Fig. 2. Introduction of NF-κB31 complexed to PLL-g-HA into LSE cells. LSE cells were cultured in CS-C complete medium containing 10% FCS. The Texas red-labeled NF-κB31 was included at a DNA concentration of 0, 1.25, 2.5, 5 μg/mL in the culture medium for 24 h. The cells were observed under a fluorescent microscopy for accumulation of Texas red-derived fluorescence.

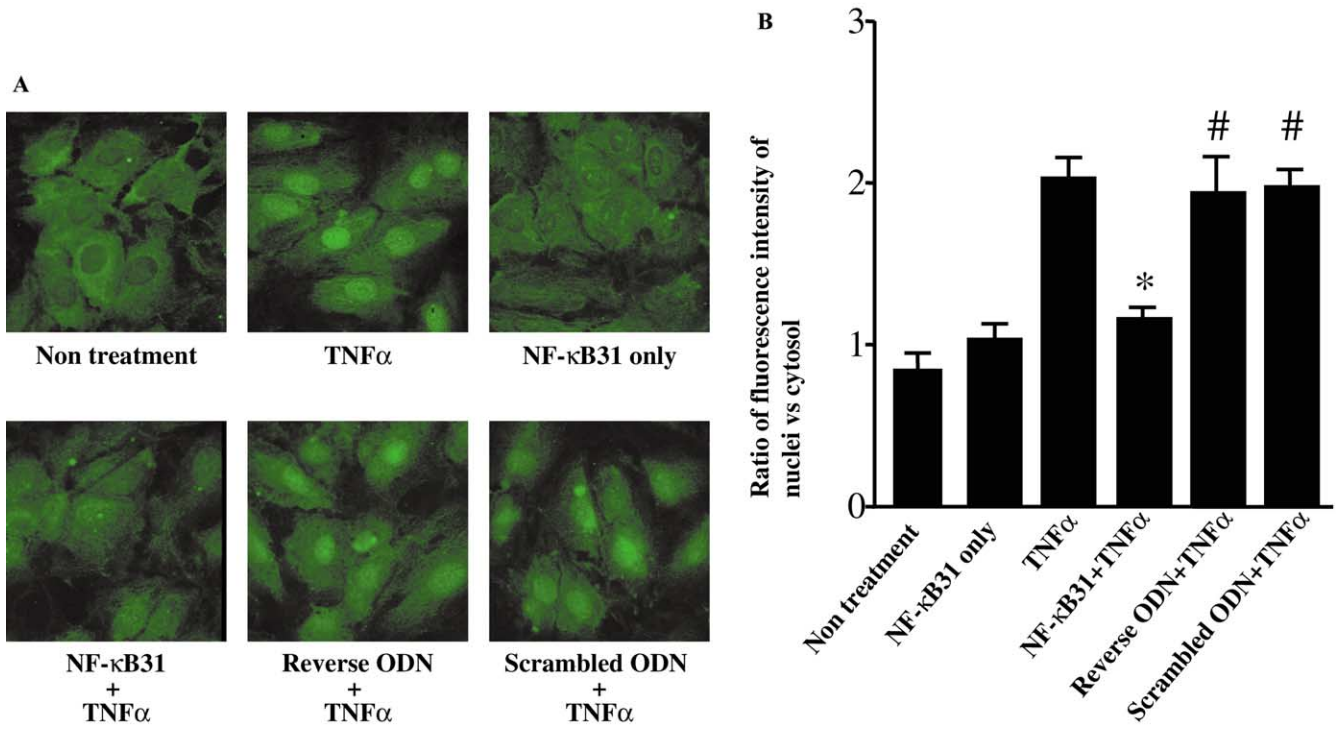


Fig. 3. Effect of NF-κB31 on TNFα-induced translocation of NF-κB in LSE cell nuclei. Conditions as in Fig. 2. LSE cells were pre-treated for 3 h with PLL-g-HA/ODN (2.5 μg/mL as DNA). Subsequently, the cells were cultured for 1 h in a new medium containing TNFα (5 ng/mL). Cells were fixed and stained for NF-κB with an antibody specific for NF-κB (p65). Representative immunostaining of NF-κB (p65) (A) and densitometric analysis (B) showing the effects of NF-κB31, Reverse ODN or Scrambled ODN on p65 nuclear level in LSE cells. The results in panel B are expressed as means ± SEM from four separate experiments. *$p < 0.01$ versus TNFα stimulated group, # ns versus TNFα stimulated group.

TNFα. Pretreatment with PLL-g-HA/NF-κB31 (2.5 μg/mL as DNA) starting 3 h before TNFα addition resulted in a marked decrease in ICAM-1 mRNA as compared to that stimulated only with TNFα (Fig. 4A). But inclusion of Reverse or Scrambled ODNs in a PLL-g-HA complexed form failed to alter the level of ICAM-1 mRNA (Fig. 4A).

After 4 h of culture with TNFα, immunostaining demonstrated a pronounced upregulation of ICAM-1 on the LSE cell membrane (Fig. 4B). As expected, ICAM-1 expression on the LSE cells was markedly diminished by PLL-g-HA/NF-κB31. This effect of NF-κB31 was sequence-specific since other control ODNs having reverse or scrambled sequences did not inhibit the TNFα-induced increase in ICAM-1 protein levels on the cell surface (Fig. 4B).

## Discussion

The decoy strategy has been developed and considered a useful tool as a new class of anti-gene strategy [11–14]. In this study, we utilized decoy ODNs having a hairpin structure, which was reportedly more resistant to DNase due to a closed end composed of a stretch of T [19]. This ODN can function as a decoy for NF-κB as shown by EMSA that demonstrated that NF-κB31 has an ability to bind to a consensus sequence for NF-κB (Fig. 1B).

We employed the PLL-g-HA vector, a non-viral gene delivery system to the SEC that is composed of a PLL backbone and chains of HA oligomers [15,16]. The polycation PLL and negatively charged DNA form a stable and condensed nanoassociate surrounded by highly hydrated HA glycocalyx. The PLL-g-HA/DNA complex is specifically taken up by SECs through an HA receptor mediated pathway since SECs possess receptors that recognize and internalize HA [21,22].

As expected, the PLL-g-HA/NF-κB31 facilitated its entry into LSE cells (Fig. 2). The bright spotty appearance indicates that the complex was predominantly localized in endosome and/or lysosome compartments. The process is HA-receptor mediated as the presence of 250× molar excess of HA inhibited the incorporation of complex almost completely (data not shown). A considerable part of the ODNs undergoes digestion through this pathway; however, the intralysozomal confinement and digestion are far from perfect and some fractions escape into cytosolic compartment [23]. Our data show that there is an increase in fluorescence intensity in the cytosol as well as in nuclei of LSE cells (Fig. 2).

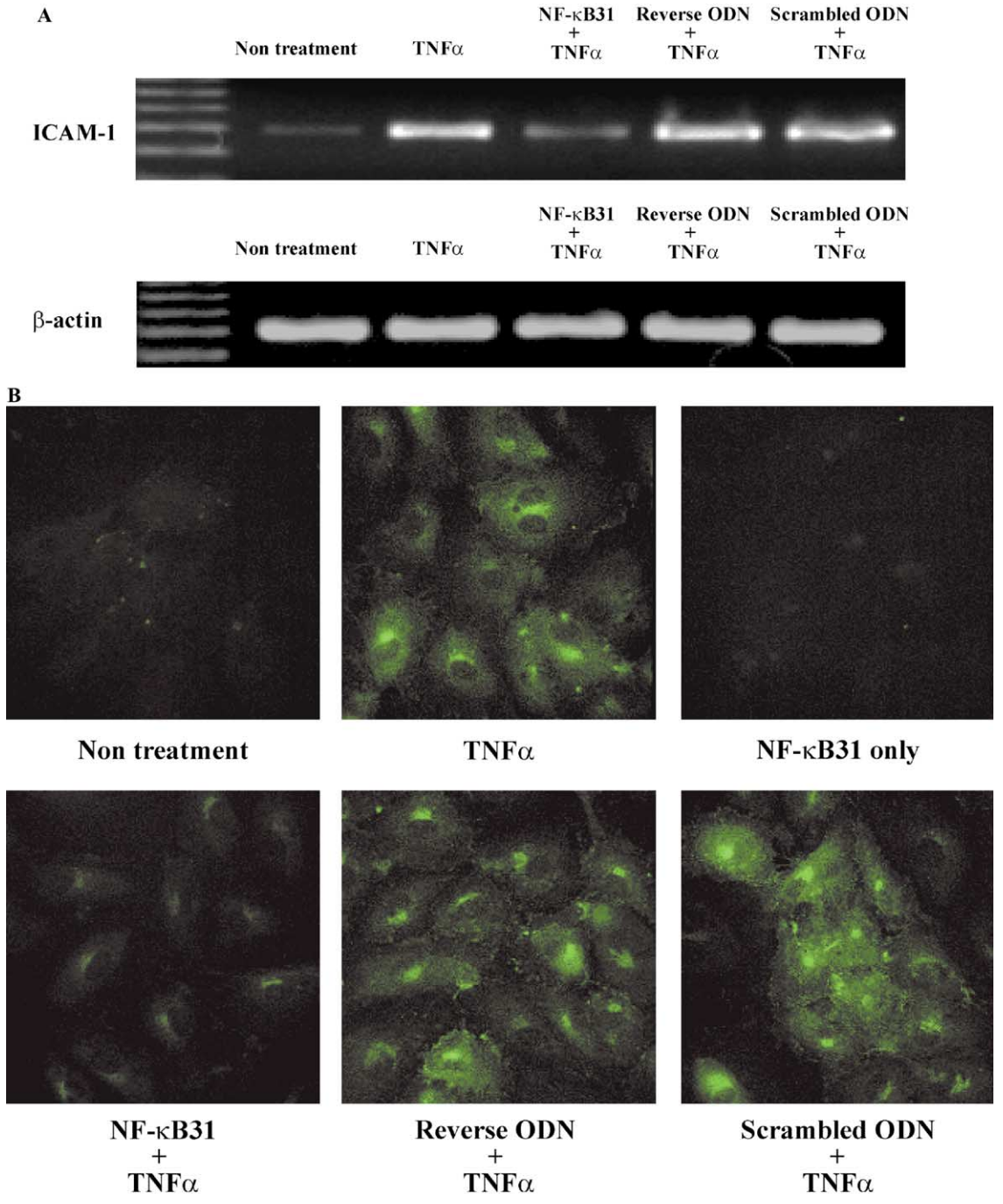


Fig. 4. Effect of PLL-g-HA/NF-κB 31 on TNFα-induced upregulation of ICAM-1 mRNA and protein. Conditions as in Fig. 2. LSE cells were pretreated for 3 h with PLL-g-HA/ODN (2.5 μg/mL as DNA). (A) The cells were cultured for 2 h in a new medium containing TNFα (5 ng/mL). Total RNA was extracted from the cells and RT-PCR was performed to determine the expression of ICAM-1 mRNA. (B) After treatment for 4 h with TNFα (5 ng/mL), LSE cells were fixed with ice-cold ethanol and incubated with a primary anti-ICAM-1 antibody. Cells were then incubated with the secondary antibody, anti-mouse goat IgG conjugated to FITC for 1 h at room temperature as described in Materials and methods.

It has been well documented that TNFα induces ICAM-1 expression in SECs through a pathway dependent on NF-κB. In principle, the presence of ODNs having consensus sequences for NF-κB should impede binding of NF-κB protein to its authentic cognate sequences in the genome thereby abrogating NF-κB-dependent gene expression. To support this notion, our data indicated that NF-κB31 effectively blocks translocation of NF-κB into cell nuclei (Fig. 3). This was not due to non-specific effects of ODNs since other two control ODNs (Reverse and Scrambled) of identical structure but lacking consensus sequences for NF-κB failed to block translocation of NF-κB. Furthermore, NF-κB31, but not Scrambled or Reverse ODN, blunted

TNFα-induced increases in ICAM-1 mRNA as well as its protein levels expressed on LSE cell membrane (Figs. 4A and B).

NF-κB is a crucial target for modulation of expression genes that are involved in various aspects of liver pathophysiology. To date, several techniques have been introduced in an attempt to block activation of NF-κB. Among them, a dominant negative form of IκB [24,25], expressed via gene transfer with viral or non-viral vectors [26,27], is widely used. The dominant negative IκB has a high binding affinity for NF-κB and barely dissociates from NF-κB even when phosphorylated [28,29]. This technique, however, has some limitations, (1) there is a considerable time needed for the dominant negative IκB gene to be expressed and (2) once the gene is expressed, it is hard to "switch off" the suppressive effect on NF-κB. The decoy system is expected to circumvent the drawbacks since it may exert its effect immediately after being introduced into cells and has a relatively short duration time of efficacy due to enzymatic degradation of ODNs. In the case sustained suppression of NF-κB action is needed, repetitive administration of decoys is possible.

As a conclusion, blocking of NF-κB activation by the NF-κB31 decoy led to inhibition of the TNFα-induced ICAM-1 expression. The decoy technique in combination with PLL-g-HA may provide a novel strategy for treatment of endotoxin-induced liver damage and prevention of transplanted graft damage in which the NF-κB-dependent ICAM-1 overexpression plays a pivotal role through the manipulation of SEC functions in vivo.

## References


[1] Y. Takei, W.S. Gao, T. Hijioka, E. Savier, K.A. Lindert, J.J. Lemasters, R.G. Thurman, Increase in survival of liver grafts after rinsing with warm Ringer's solution due to improvement of hepatic microcirculation, Transplantation 52 (1991) 225–230.

[2] A. Mantovani, F. Bussolino, E. Dejana, Cytokine regulation of endothelial cell function, FASEB J. 6 (1992) 2591–2599.

[3] J.S. Pober, R.S. Cotran, The role of endothelial cells in inflammation, Transplantation 50 (1990) 537–544.

[4] K. Ikejima, Y. Takei, H. Honda, M. Hirose, M. Yoshikawa, Y.J. Zhang, T. Lang, T. Fukuda, S. Yamashina, T. Kitamura, N. Sato, Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat, Gastroenterology 122 (2002) 1399–1410.

[5] F.H. Bach, S.C. Robson, H. Winkler, C. Ferran, K.M. Stuhlmeier, C.J. Wrighton, W.W. Hancock, Barriers to xenotransplantation, Nat. Med. 1 (1995) 869–873.

[6] M.W. Hoffmann, K. Wonigeit, G. Steinhoff, H. Herzbeck, H.D. Flad, R. Pichlmayr, Production of cytokines (TNF-α, IL-1-β) and endothelial cell activation in human liver allograft rejection, Transplantation 55 (1993) 329–335.

[7] A.S. Baldwin Jr., The NF-κB and IκB proteins: new discoveries and insights, Annu. Rev. Immunol. 14 (1996) 649–683.

[8] F.H. Bach, S.C. Robson, C. Ferran, H. Winkler, M.T. Millan, K.M. Stuhlmeier, B. Vanhove, M.L. Blakely, W.J. van der Werf, E. Hofer, R. de Martin, W.W. Hancock, Endothelial cell activation and thromboregulation during xenograft rejection, Immunol. Rev. 141 (1994) 5–30.

[9] P.A. Baeuerle, T. Henkel, Function and activation of NF-κB in the immune system, Annu. Rev. Immunol. 12 (1994) 141–179.

[10] I.M. Verma, J.K. Stevenson, E.M. Schwarz, D. van Antwerp, S. Miyamoto, Rel/NF-κB/IκB family: intimate tales of association and dissociation, Genes Dev. 9 (1995) 2723–2735.

[11] A. Bielinska, R.A. Shivdasani, L.Q. Zhang, G.J. Nabel, Regulation of gene expression with double stranded phosphorothioate oligonucleotides, Science 250 (1990) 997–1000.

[12] R. Morishita, M. Aoki, Y. Kaneda, Decoy oligodeoxynucleotides as novel cardiovascular drugs for cardiovascular disease, Ann. N Y Acad. Sci. 947 (2001) 294–301.

[13] R. Morishita, J. Higaki, N. Tomita, T. Ogihara, Application of transcription factor "decoy" strategy as means of gene therapy and study of gene expression in cardiovascular disease, Circ. Res. 82 (1998) 1023–1028.

[14] N. Tomita, R. Morishita, J. Higaki, T. Ogihara, A novel strategy for gene therapy and gene regulation analysis using transcription factor decoy oligonucleotides, Exp. Nephrol. 5 (1997) 429–434.

[15] S. Asayama, M. Nogawa, Y. Takei, T. Akaike, A. Maruyama, Synthesis of novel polyampholyte comb-type copolymers consisting of a poly(L-lysine) backbone and hyaluronic acid side chains for a DNA carrier, Bioconjug. Chem. 9 (1998) 476–481.

[16] Y. Takei, A. Maruyama, S. Kawano, Y. Nishimura, S. Asayama, M. Nogawa, K. Ikejima, M. Hori, T. Akaike, J.J. Lemasters, S. Watanabe, N. Sato, Targeted gene transfer to sinusoidal endothelial cells and expression in vivo, Transplant. Proc. 31 (1999) 790–791.

[17] G. Nabel, D. Baltimore, An inducible transcription factor activates expression of human immunodeficiency virus in T cell, Nature 326 (1987) 711–713.

[18] M. Grilli, J.J. Chiu, M.J. Lenardo, NF-κB and Rel: participants in a multiform transcriptional regulatory system, Int. Rev. Cytol. 143 (1993) 1–62.

[19] A.V. Maksimenko, M.B. Gottikh, V. Helin, Z.A. Shabarova, C. Malvy, Physico-chemical and biological properties of antisense phosphodiester oligonucleotides with various secondary structures, Nucleosides Nucleotides 18 (1999) 2071–2091.

[20] J.D. Dignam, R.M. Lebovitz, R.G. Roeder, Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei, Nucleic Acids. Res. 11 (1983) 1475–1489.

[21] N. Forsberg, S. Gustafson, Characterization and purification of the hyaluronan-receptor on liver endothelial cells, Biochim. Biophys. Acta 30 (1991) 12–18.

[22] J. Yannariello-Brown, S.J. Frost, P.H. Weigel, Identification of the $Ca^{2+}$-independent endocytic hyaluronan receptor in rat liver sinusoidal endothelial cells using a photoaffinity cross-linking reagent, J. Biol. Chem. 267 (1992) 20451–20456.

[23] G.Y. Wu, C.H. Wu, Specific inhibition of hepatitis B viral gene expression in vitro by targeted antisense oligonucleotides, J. Biol. Chem. 267 (1992) 12436–12439.

[24] A.A. Beg, D. Baltimore, An essential role for NF-κB in preventing TNF-α-induced cell death, Science 274 (1996) 782–784.

[25] Z.G. Liu, H. Hsu, D.V. Goeddel, M. Karin, Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death, Cell 87 (1996) 565–576.

[26] C. Ferran, D.M. Stroka, A.Z. Badrichani, J.T. Cooper, F.H. Bach, Adenovirus-mediated gene transfer of A20 renders endothelial cells resistant to activation: a means of evaluating the role of endothelial cell activation in xenograft rejection, Transplant. Proc. 29 (1997) 879–880.

[27] C. Jobin, A. Panja, C. Hellerbrand, Y. Iimuro, J. Didonato, D.A. Brenner, R.B. Sartor, Inhibition of proinflammatory molecule production by adenovirus-mediated expression of a nuclear factor

κB super-repressor in human intestinal epithelial cells, J. Immunol. 160 (1998) 410–418.
[28] T.R. Bakker, T. Renno, C.V. Jongeneel, Impaired fetal thymocyte development after efficient adenovirus-mediated inhibition of NF-κB activation, J. Immunol. 162 (1999) 3456–3462.
[29] C.J. Wrighton, R. Hofer-Warbinek, T. Moll, R. Eytner, F.H. Bach, R. de Martin, Inhibition of endothelial cell activation by adenovirus-mediated expression of IκBα, an inhibitor of the transcription factor NF-κB, J. Exp. Med. 183 (1996) 1013–1022.