

Short
CommunicationEffective treatment of retrovirus-induced
suppression of antibody responses with CpG
oligodeoxynucleotidesAnke R. M. Kraft,¹ Tanja Arndt,¹ Kim J. Hasenkrug² and Ulf Dittmer¹

Correspondence

Ulf Dittmer

ulf.dittmer@uni-essen.de

¹Institut für Virologie des Universitätsklinikums Essen, Hufelandstrasse 55, 45122 Essen, Germany²Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

Most retroviruses induce severe immunosuppression during acute infection. We have used the Friend retrovirus mouse model to demonstrate that immunostimulatory B-type CpG oligodeoxynucleotides (ODN) have a protective effect against retrovirus-induced suppression of antibody responses to potent B-cell antigens. CD8⁺ T cells were critical for effective treatment with CpG-ODN, since *in vivo* depletion of these cells from treated mice impaired protection from retrovirus-induced immunosuppression. Protection also required IFN- γ , as neutralization of this cytokine abolished the therapeutic effect of CpG-ODN. These findings may have implications for the treatment of immunosuppressive virus infections.

Received 18 April 2005

Accepted 18 September 2005

Many viruses induce immunosuppression during acute or chronic infection. This immunosuppression is often associated with severe secondary infections with other pathogens. For example, retroviruses such as human immunodeficiency virus (HIV) and human T-lymphotropic virus 1 can induce immunosuppression in the human host, which leads to life-threatening clinical symptoms. In the current study, we have used the Friend virus (FV) mouse model to develop new therapeutic approaches that can prevent immunosuppression during an acute retroviral infection. FV is a viral complex comprising two retroviruses: the replication-competent helper virus called Friend murine leukemia virus, which is non-pathogenic in adult mice, and the replication-defective but pathogenic spleen focus-forming virus (Kabat, 1989). Infection of adult mice with FV complex induces acute splenomegaly due to rapid polyclonal erythroblast proliferation, which is followed by the development of lethal erythroleukaemia. In addition to erythroleukaemia, certain strains of mice develop a severe immunosuppression during acute FV infection characterized by impaired antibody responses to potent B-cell antigens, such as sheep red blood cells (SRBC) (Faxvaag *et al.*, 1995; Dittmer *et al.*, 2002). The severity of FV-induced immunosuppression is influenced by the H-2D class I gene region of the MHC (Chesebro *et al.*, 1990). Experiments with MHC-recombinant mice showed that homozygous H-2D^{a/a} mice were unable to mount antibody responses to SRBC during acute FV infection. In contrast, mice with two b alleles at this locus were resistant to FV-mediated suppression of antibody responses (Dittmer *et al.*, 2002). Heterozygous H-2D^{a/b} mice generated antibody responses to SRBC during acute FV infection but these were severely suppressed compared with SRBC-immunized

uninfected mice (Fig. 1). We have previously reported that the association between the H-2D haplotype of mice and immunosuppression can be explained by the finding that H-2D^b-restricted, IFN- γ -producing CD8⁺ T cells protect mice against FV-induced suppression of antibody responses (Dittmer *et al.*, 2002). Thus, an immunotherapeutic approach that augments CD8⁺ T-cell responses during acute FV infection should prevent or reduce retrovirus-induced immunosuppression. From recent experiments, we knew that post-exposure treatment of acute FV-infected mice with synthetic oligodeoxynucleotides (ODN) containing immunostimulatory CpG motifs (CpG-ODN) induced a strong Th-1-dominated cytokine milieu (high IFN- γ production) and increased numbers of FV-specific CD8⁺ T cells, which protected mice from virus-induced leukaemia (Olbrich *et al.*, 2002). In addition, *in vivo* treatment of mice with CpG-ODN has also been shown to activate antigen-presenting cells that then promote IFN- γ production by T cells and induction of antigen-specific cytotoxic T cells (Krieg, 2001; Olbrich *et al.*, 2002; Sun *et al.*, 1998; Walker *et al.*, 1999). Furthermore, CpG-ODN can enhance antibody production by B cells (Krieg *et al.*, 1995; Yamamoto *et al.*, 1992). Thus, CpG-ODN should be a promising therapeutic agent to treat virus-induced suppression of antibody responses during acute retroviral infections.

To evaluate the effect of CpG-ODN on FV-induced suppression of antibody responses, FV-susceptible H-2^{a/b} mice were infected with 3000 spleen focus-forming units (s.f.f.u.) FV. During acute infection, mice were treated with 15 nmol of the B-type CpG-ODN (CpG-1668; 5'-TCCATGACGTTCCCTGATGCT-3') or control ODN

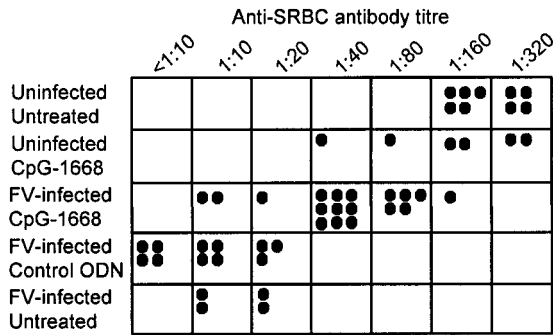


Fig. 1. Effect of CpG-ODN treatment on FV-induced suppression of antibody responses. Plasma samples from H-2D^{a/b} (A.BY × B10.A) F₁ mice were tested for anti-SRBC antibody titres at 21 days after infection with 3000 s.f.u. FV (Dittmer *et al.*, 2002). Mice were treated intraperitoneally with a B-type CpG-ODN (CpG-1668) or control ODN (15 nmol per mouse) on days 5 and 13 post-infection. The differences between geometric mean (log₂) anti-SRBC titres in infected, control ODN-treated mice and uninfected, untreated or CpG-ODN-treated mice were statistically significant ($P < 0.001$). The difference between geometric mean (log₂) anti-SRBC titres in infected, control ODN-treated mice and infected, CpG-ODN-treated mice was also statistically significant ($P < 0.001$). No differences in anti-SRBC antibody titres were observed between FV-infected, control ODN-treated and FV-infected, non-treated mice. Data from two independent experiments were combined.

(5'-TCCATGAGCTTCCTGATGCT-3') at 5 and 13 days post-infection (Olbrich *et al.*, 2003). At 14 days post-infection, all mice were immunized intraperitoneally with SRBC and then tested 1 week later for antibody responses to SRBC using a haemagglutination assay (Dittmer *et al.*, 2002). Statistical significance of results was determined throughout by one-way analysis of variance using Dunnett's multiple-comparison correction.

Heterozygous H-2D^{a/b} mice inoculated with control ODN after FV infection had significantly suppressed antibody titres (mean 1:5) against SRBC compared with uninfected, untreated control mice (mean 1:199) (Fig. 1). In contrast, FV-infected mice that were treated with CpG-ODN showed significantly higher antibody titres (mean 1:43) than the group of animals that received the control ODN (mean 1:5). The induced antibodies against SRBC were of both IgM and IgG subtypes (data not shown). Thus, the CpG-ODN treatment significantly reduced FV-induced suppression of antibody responses in mice. These results indicate the potential of CpG-ODN to treat retrovirus-induced immunosuppression.

To determine whether CD8⁺ T cells were required for the therapeutic effect of CpG-ODN in H-2D^{a/b} mice, CD8⁺ T cells were depleted in FV-infected, CpG-treated animals using monoclonal antibodies (mAbs) (Hasenkrug *et al.*, 1998). CD8⁺ T-cell-depleted mice developed severe

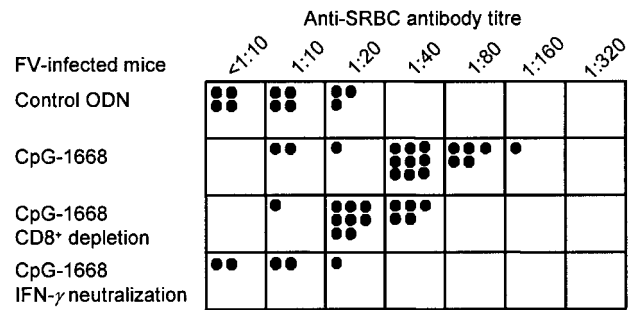


Fig. 2. CpG-ODN-mediated reduction of FV-induced suppression of antibody responses was CD8⁺ T cell and IFN- γ dependent. Plasma samples from H-2D^{a/b} (A.BY × B10.A) F₁ mice were tested for anti-SRBC antibody titres at 21 days after infection with 3000 s.f.u. FV (Dittmer *et al.*, 2002). Mice were treated intraperitoneally with CpG-ODN (CpG-1668; 15 nmol per mouse) on days 5 and 13 post-infection. CD8⁺ T-cell depletions were performed by five intraperitoneal injections (days 0, 2, 5, 7 and 13 post-infection) of supernatant from clone 169.4 mAb starting at the time of infection. At 8 days following the last injection of anti-CD8 antibodies, splenic CD8⁺ T cells were reduced to less than 1.44% (mean of 15.8% in non-depleted mice) of nucleated spleen cells. The difference between geometric mean (log₂) anti-SRBC titres in infected and CpG-ODN-treated mice and CD8⁺ T-cell-depleted, infected and CpG-ODN-treated mice was statistically significant ($P < 0.05$). IFN- γ neutralization was done by five intraperitoneal injections of rat mAb XMG1.2 starting at the time of infection (day 0, 2, 5, 9 and 13 post-infection). The difference between geometric mean (log₂) anti-SRBC titres in infected and CpG-ODN-treated mice and IFN- γ -neutralized, infected and CpG-ODN-treated mice was statistically significant ($P < 0.002$). The data for CpG-ODN- and control CpG-treated mice were combined from the same two independent experiments as shown in Fig. 1. Isotype controls for both antibodies were used in a previous study but did not have any effect on FV-induced suppression of anti-SRBC antibody responses (Dittmer *et al.*, 2002).

FV-induced suppression of antibody responses despite CpG-ODN inoculation (Fig. 2), indicating that CD8⁺ T cells were involved in the CpG-mediated protection against suppression of humoral immunity. An important function of CD8⁺ T cells which affects suppression of antibody responses during acute FV infection is the production of IFN- γ (Dittmer *et al.*, 2002). To analyse the potential effect of IFN- γ on CpG-induced protection against suppression of anti-SRBC responses, IFN- γ was neutralized by injection of anti-IFN- γ antibodies during acute FV infection (Dittmer *et al.*, 2002). The neutralization of IFN- γ in infected, CpG-treated mice significantly reduced SRBC antibody titres in comparison with those of the infected, CpG-treated control group. Thus, IFN- γ was an essential component of the CpG-mediated protection against immunosuppression during acute FV infection. Interestingly, IFN- γ neutralization in FV-infected, CpG-ODN-treated mice resulted in a stronger

reduction of SRBC antibody responses than CD8⁺ T-cell depletion (Fig. 2). This implies that cell types other than CD8⁺ T cells may also contribute to the production of protective IFN- γ .

The protective effects of CD8⁺ T cells and IFN- γ on retrovirus-mediated suppression of antibody responses were independent of virus loads in the treated animals, as the two treatments influenced infection levels in these mice differently. Whereas the depletion of CD8⁺ T cells slightly increased the number of infected spleen cells in CpG-treated, FV-infected mice, neutralization of IFN- γ decreased spleen virus loads in such animals compared with CpG-treated, FV-infected mice (Fig. 3). However, these differences in virus loads were not statistically significant. These findings confirmed previous results obtained with IFN- γ -knockout mice, which were more effective in controlling FV replication during acute infection than wild-type mice (Stromnes *et al.*, 2002). The current studies indicate that protection against retrovirus-induced suppression of antibody responses by CpG-ODN was mediated by CD8⁺ T cells and IFN- γ but was independent of replication levels during acute FV infection. *In vitro* studies with lymphocytes from simian immunodeficiency virus (SIV)-infected macaques or HIV-infected humans previously implied that CpG-ODN might be an effective therapeutic agent against retroviral infections (Teleshova *et al.*, 2004; Schlaepfer *et al.*, 2004). Teleshova *et al.* (2004) showed that CpG-ODN could

activate lymphocytes and dendritic cells of SIV-infected macaques in cell culture, suggesting a possible therapeutic approach to overcome virus-mediated immunodeficiency. On the other hand, CpG-ODN have also been reported to reactivate persistent HIV *in vitro* (Equils *et al.*, 2003; Scheller *et al.*, 2004). Thus, the outcome of a CpG-ODN treatment of infected patients could not be predicted from such experiments. Initial evidence that CpG-ODN might be effective *in vivo* to treat retrovirus-induced immunosuppression came from the SIV-macaque model of HIV. In two interesting papers, Verthelyi and colleagues reported that SIV-infected monkeys developed antibody responses to a hepatitis B vaccine (Verthelyi *et al.*, 2004) or were partially protected from leishmania superinfection (Verthelyi *et al.*, 2003) after CpG-ODN treatment. This was in sharp contrast to SIV-infected, untreated monkeys, which did not mount antibody responses (Verthelyi *et al.*, 2004) and developed progressive cutaneous lesions after leishmania challenge (Verthelyi *et al.*, 2003). Thus, CpG-ODN had the potential to overcome at least partially the severe immunosuppressive effects of SIV *in vivo*. We have now extended these studies and report that CpG-ODN also protect mice against FV-induced suppression of antibody responses. The utilization of mice for these experiments enabled us to demonstrate that CD8⁺ T cells and IFN- γ were critically involved in the protective effect of CpG-ODN. Since there is no evidence that CpG-ODN can stimulate T cells directly (Hornung *et al.*, 2002), the most likely scenario was that the CpG-ODN stimulated maturation of dendritic cells, which then activated T cells to produce IFN- γ (Teleshova *et al.*, 2004). In fact, it has been reported that dendritic cells from HIV-infected patients can be matured *in vitro* by immunostimulatory CpG-ODN (Verthelyi *et al.*, 2003). These current findings might provide new strategies to treat immunosuppression induced by retroviruses or other virus infections.

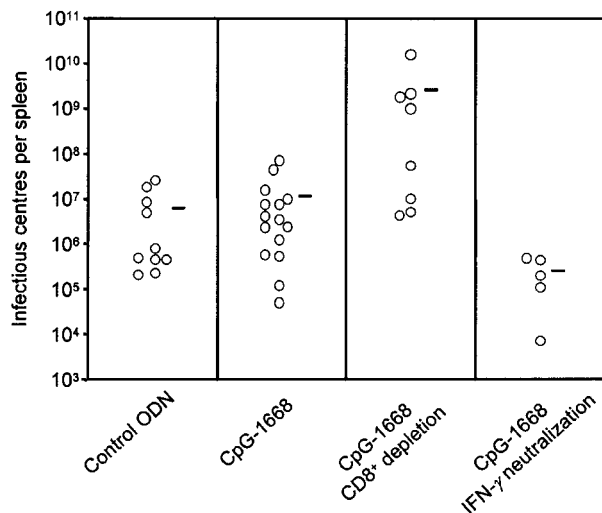


Fig. 3. Levels of FV infection in the spleens of CpG-ODN-treated mice. Spleen cells from mice taken at 3 weeks post-infection were used to determine levels of FV infection. Mice were treated in the acute phase of infection intraperitoneally with CpG-ODN or control ODN (15 nmol per mouse) on days 5 and 13 post-infection. The differences between the CpG-ODN-treated group and the group of mice that were depleted for CD8⁺ T cells or in which IFN- γ was neutralized were not statistically significant ($P > 0.05$). Bars represent the mean number of infectious centres per spleen of each group.

Acknowledgements

The work was supported by grants to U.D. from the Deutsche Forschungsgemeinschaft (Di 714/6-1 and Di 714/6-2).

References

- Chesebro, B., Miyazawa, M. & Britt, W. J. (1990). Host genetic control of spontaneous and induced immunity to Friend murine retrovirus infection. *Annu Rev Immunol* 8, 477–499.
- Dittmer, U., Race, B., Peterson, K. E., Stromnes, I. M., Messer, R. J. & Hasenkrug, K. J. (2002). Essential roles for CD8⁺ T cells and gamma interferon in protection of mice against retrovirus-induced immunosuppression. *J Virol* 76, 450–454.
- Equils, O., Schito, M. L., Karahashi, H., Madak, Z., Yarali, A., Michelsen, K. S., Sher, A. & Arditi, M. (2003). Toll-like receptor 2 (TLR2) and TLR9 signaling results in HIV-long terminal repeat *trans*-activation and HIV replication in HIV-1 transgenic mouse spleen cells: implications of simultaneous activation of TLRs on HIV replication. *J Immunol* 170, 5159–5164.
- Faxvaag, A., Espevik, T. & Dalen, A. (1995). An immunosuppressive murine leukaemia virus induces a Th1→Th2 switch and abrogates

- the IgM antibody response to sheep erythrocytes by suppressing the production of IL-2. *Clin Exp Immunol* **102**, 487–495.
- Hasenkrug, K. J., Brooks, D. M. & Dittmer, U. (1998).** Critical role for CD4⁺ T cells in controlling retrovirus replication and spread in persistently infected mice. *J Virol* **72**, 6559–6564.
- Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., Jahrsdorfer, B., Giese, T., Endres, S. & Hartmann, G. (2002).** Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol* **168**, 4531–4537.
- Kabat, D. (1989).** Molecular biology of Friend viral erythroleukemia. *Curr Top Microbiol Immunol* **148**, 1–42.
- Krieg, A. M. (2001).** Now I know my CpGs. *Trends Microbiol* **9**, 249–252.
- Krieg, A. M., Yi, A. K., Matson, S., Waldschmidt, T. J., Bishop, G. A., Teasdale, R., Koretzky, G. A. & Klinman, D. M. (1995).** CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* **374**, 546–549.
- Olbrich, A. R. M., Schimmer, S., Heeg, K., Schepers, K., Schumacher, T. N. & Dittmer, U. (2002).** Effective postexposure treatment of retrovirus-induced disease with immunostimulatory DNA containing CpG motifs. *J Virol* **76**, 11397–11404.
- Olbrich, A. R. M., Schimmer, S. & Dittmer, U. (2003).** Preinfection treatment of resistant mice with CpG oligodeoxynucleotides renders them susceptible to Friend retrovirus-induced leukemia. *J Virol* **77**, 10658–10662.
- Scheller, C., Ullrich, A., McPherson, A. & 10 other authors (2004).** CpG oligodeoxynucleotides activate HIV replication in latently infected human T cells. *J Biol Chem* **279**, 21897–21902.
- Schlaepfer, E., Audige, A., von Beust, B., Manolova, V., Weber, M., Joller, H., Bachmann, M. F., Kundig, T. M. & Speck, R. F. (2004).** CpG oligodeoxynucleotides block human immunodeficiency virus type 1 replication in human lymphoid tissue infected ex vivo. *J Virol* **78**, 12344–12354.
- Stromnes, I. M., Dittmer, U., Schumacher, T. N., Schepers, K., Messer, R. J., Evans, L. H., Peterson, K. E., Race, B. & Hasenkrug, K. J. (2002).** Temporal effects of gamma interferon deficiency on the course of Friend retrovirus infection in mice. *J Virol* **76**, 2225–2232.
- Sun, S., Kishimoto, H. & Sprent, J. (1998).** DNA as an adjuvant: capacity of insect DNA and synthetic oligodeoxynucleotides to augment T cell responses to specific antigen. *J Exp Med* **187**, 1145–1150.
- Teleshova, N., Kenney, J., Jones, J. & 7 other authors (2004).** CpG-C immunostimulatory oligodeoxyribonucleotide activation of plasmacytoid dendritic cells in rhesus macaques to augment the activation of IFN-gamma-secreting simian immunodeficiency virus-specific T cells. *J Immunol* **173**, 1647–1657.
- Verthelyi, D., Gursel, M., Kenney, R. T., Lifson, J. D., Liu, S., Mican, J. & Klinman, D. M. (2003).** CpG oligodeoxynucleotides protect normal and SIV-infected macaques from Leishmania infection. *J Immunol* **170**, 4717–4723.
- Verthelyi, D., Wang, V. W., Lifson, J. D. & Klinman, D. M. (2004).** CpG oligodeoxynucleotides improve the response to hepatitis B immunization in healthy and SIV-infected rhesus macaques. *AIDS* **18**, 1003–1008.
- Walker, P. S., Scharton-Kersten, T., Krieg, A. M., Love-Homan, L., Rowton, E. D., Udey, M. C. & Vogel, J. C. (1999).** Immunostimulatory oligodeoxynucleotides promote protective immunity and provide systemic therapy for leishmaniasis via IL-12- and IFN-gamma-dependent mechanisms. *Proc Natl Acad Sci U S A* **96**, 6970–6975.
- Yamamoto, S., Yamamoto, T., Shimada, S., Kuramoto, E., Yano, O., Kataoka, T. & Tokunaga, T. (1992).** DNA from bacteria, but not from vertebrates, induces interferons, activates natural killer cells and inhibits tumor growth. *Microbiol Immunol* **36**, 983–997.