Commentary

Oligonucleotide Adjuvants for T Helper 1 (Th1)–specific Vaccination

By Dennis A. Carson and Eyal Raz

From the Sam and Rose Stein Institute for Research on Aging and the Department of Medicine, University of California, San Diego, La Jolla, California 92093-0663

T helper cell 1 (Th1)-dependent delayed hypersensitivity reactions are an important part of host defenses against intracellular infections. Yet, more than two centuries after Jenner's successful inoculation against smallpox, we still do not know exactly how to produce safe vaccines that stimulate Th1 immunity. Specialized bone marrow–derived antigen-presenting cells normally are required to initiate all T cell–dependent immune responses (1). However, in order for Th responses to shift to a Th1 phenotype, interleukin (IL)-12 needs to be present at the time of antigen recognition (2). IL-12 drives natural killer (NK) and Th1 cells to generate interferon (IFN)-γ, that subsequently impels macrophages to initiate delayed hypersensitivity reactions. IFN-γ also inhibits the synthesis of IL-4 and IL-5 by Th2 cells (3). In the absence of IL-12 induced IFN-γ production, Th2 responses usually dominate.

But how do particular infectious agents induce IL-12 release? Accumulating evidence indicates that immunostimulatory CpG sequences (ISS) in the DNA of bacteria may be one of the major IL-12–inducing factors (4). Complete Freund's adjuvant, composed of killed mycobacteria dispersed in mineral oil, is an established inducer of Th1-dependent delayed hypersensitivity reactions. More than 10 years ago, Tokunaga and coworkers discovered that DNA purified from mycobacteria fostered the release of IFN-γ by mouse NK cells (5). Fractionation of the DNA led to the isolation of several different short palindromic sequences, most of them centered around a CpG dinucleotide core, that had direct NK stimulatory activity (6). Subsequent studies showed that synthetic phosphodiester or phosphothioate oligodeoxynucleotides, which reproduced the immunostimulatory DNA sequences from mycobacteria, could activate NK cells and induce B lymphocyte proliferation in vitro (6–9). Methylation of cytosine residues in the bacterial DNA or in the corresponding oligodeoxynucleotides destroyed their immunostimulatory activities (7).

During early investigations of DNA vaccination, we observed that nonspecific bacterial DNA enhanced immune responses to a coinjected antigen expression vector (10). Naked DNA immunization stimulated a selective Th1 immune response that persisted upon secondary challenge with protein antigen (11). In some instances, the Th1 skewing effects of gene vaccines could be manipulated by changing the number of immunostimulatory sequences in the plasmid DNA backbone (12). Simple coinjection of bacterial DNA or immunostimulatory oligodeoxynucleotides with a DNA vaccine or with representative protein antigens also promoted antigen-specific Th1 responses (13, 14), even in mice with preexistent Th2 immunity. Incubation of purified human macrophages with bacterial DNA, or with immunostimulatory CpG oligodeoxynucleotides, stimulated the production of IL-12, IL-18, and IFN-α (14).

Now, Chu et al. have shown that vaccination of mice with an antigen and an immunostimulatory CpG oligodeoxynucleotide in incomplete Freund's adjuvant induced a powerful Th1 immune response, comparable to that achieved by coinjection of the antigen in complete Freund's adjuvant (15). In contrast, mice vaccinated with antigen and control oligodeoxynucleotides lacking the CpG motif, developed a skewed Th2 type immune response.

An efficacious vaccine must be devoid of systemic toxicity. Systemically administered immunostimulatory oligodeoxynucleotides can trigger a cytokine syndrome in mice, characterized by TNF release, hypotension, and shock (16). Although exogenous IL-12 induces potent Th1 immune responses, high concentrations of the cytokine also can be harmful to the recipient (17). The potential side effects of immunostimulatory CpG sequences could be reduced by including them in the backbones of DNA vaccines, or by tethering the immunostimulatory oligodeoxynucleotides directly to precipitated antigens.

We still do not understand how mouse macrophages, B lymphocytes, and NK cells recognize specific DNA sequences in bacterial DNA. The immunostimulatory CpG motifs could theoretically bind to complementary sequences in DNA or mRNA. More likely, the unmethylated CpG core interacts with one or more signal transduction molecules in the cytoplasm, or on the plasma membrane. An analogous system mediates the induction of IFN-α synthesis by double stranded viral RNA (18).

In the future, it may be possible to skew the immune response to vaccination to a Th1, Th2, or a mixed Th1/Th2 outcome, simply by titering the concentrations of coadministered immunostimulatory CpG oligodeoxynucleotides. Th1 vaccines should be particularly useful for the prevention and treatment of allergic diseases and asthma, since the IFN-γ released by Th1 lymphocytes and NK cells can downregulate IgE synthesis, as well as inhibit Th2 cells that control the late phase component of the allergic response. It is conceivable that the coadministration of an immunostimulatory oligodeoxynucleotide sequence with a weak tumor antigen could stimulate a delayed hypersensitivity response

J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/11/1621/02 $2.00
Volume 186, Number 10, November 17, 1997 1621–1622
http://www.jem.org

sufficient to eliminate malignant cells. By increasing endogenous IFN-γ synthesis, therapeutic Th1 vaccines could promote recovery from chronic viral or parasitic infections.

In summary, immunostimulatory oligonucleotides are adjuvants that simplify Th1 induction in experimental systems. Their applications in clinical immunology will depend on whether the data generated in murine models will be reproducible in humans and whether the side effects of cytokine overproduction will be acceptable.

Received for publication 30 September 1997 and in revised form 2 October 1997.

References

1. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9:271–296.
2. Seder, R.A., and W.E. Paul. 1994. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu. Rev. Immunol.* 12:635–675.
3. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251–276.
4. Klinman, D.M., A.K. Yi, S.L. Beaucage, J. Conover, and A.M. Kreig. 1996. CpG motifs present in bacterial DNA rapidly induced lymphocytes to secrete interleukin 6, interleukin 12, and interferon gamma. *Proc. Natl. Acad. Sci. USA.* 93: 2879–2883.
5. Shimada, S., O. Yano, and T. Tokunaga. 1986. In vivo augmentation of natural killer cell activity with a deoxyribonucleic acid fraction of BCG. *Jpn. J. Cancer Res.* 77:808–816.
6. Yamamoto, S., T. Yamamoto, T. Kataoka, E. Kuramoto, O. Yano, and T. Tokunaga. 1992. Unique palindromic sequences in synthetic oligonucleotides are required to induce IFN and augment IFN-mediated natural killer activity. *J. Immunol.* 148:4072–4076.
7. Kreig, A.M., A.-K. Yi, S. Matson, T.J. Waldschmidt, G.A. Bishop, R. Teasdale, G.A. Koretsky, and D.M. Klinman. 1995. CpG motifs in bacterial DNA trigger direct B cell activation. *Nature (Lond.).* 374:546–549.
8. Liang, H., Y. Nishioka, C.F. Reich, D.S. Pisetsky, and P.E. Lipsky. 1996. Activation of human B cells by phosphorothioate oligonucleotides. *J. Clin. Invest.* 98:1119–1129.
9. Messina, J.P., G.S. Gilkeson, and D.S. Pisetsky. 1991. Stimulation of in vitro murine lymphocyte proliferation by bacterial DNA. *J. Immunol.* 147:1759–1764.
10. Raz, E., D.A. Carson, S.E. Parker, T.B. Parr, A.M. Abai, G. Aichinger, S.H. Gromkowski, M. Singh, D. Lew, M.E. Yankauckas, S.M. Baird, and G.H. Rhodes. 1994. Intradermal gene immunization: The possible role of DNA uptake in the induction of cellular immunity to viruses. *Proc. Natl. Acad. Sci. USA.* 91:9519–9523.
11. Raz, E., H. Tighe, Y. Sato, M. Corr, J. Dudler, M. Roman, S.L. Swain, H.L. Spiegelberg, and D.A. Carson. 1996. Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. *Proc. Natl. Acad. Sci. USA.* 93:5141–5145.
12. Sato, Y., M. Roman, H. Tighe, D. Lee, M. Corr, M.-D. Nguyen, G.J. Silverman, M. Lotz, D.A. Carson, and E. Raz. 1996. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science (Wash. DC).* 273:352–354.
13. Klinman, D.M., G. Yamshchikov, and Y. Ishigatsubo. 1997. Contribution of CpG motifs to the immunogenicity of DNA vaccines. *J. Immunol.* 158:3635–3639.
14. Roman, M., E. Martin-Orozco, J.S. Goodman, M.-D. Nguyen, Y. Sato, A. Ronaghy, R.S. Kornbluth, D.D. Richman, D.A. Carson and E. Raz. 1997. Immunostimulatory DNA sequences function as T helper-1 promoting adjuvants. *Nature Med.* 3:849–854.
15. Chu, R.S., O.S. Targoni, A.M. Krieg, P.V. Lehmann, and C.V. Harding. 1997. CpG oligodeoxynucleotides act as adjuvants that switch on TH immunity. *J. Exp. Med.* 186:1623–1631.
16. Sparwasser, T., T. Miethke, G. Lipford, K. Borschert, H. Haecker, K. Heeg, and H. Wagner. 1997. Bacterial DNA causes septic shock. *Nature (Lond.).* 386:336–337.
17. Lotze, M.T., L. Zitvogel, R. Campbell, P.D. Elder, C. Haluszcak, D. Martin, W.J. Starkus, and H. Tahara. 1996. Cytokine gene therapy of cancer using interleukin-12: murine and clinical trials. *Ann. NY Acad. Sci.* 795:440–454.
18. Lampson, G.P., A.A. Tytell, A.K. Field, M.M. Nemes, and M.R. Hilleman. 1967. Inducers of interferon and host resistance. I. Double-stranded RNA from extracts of Penicillium funiculosum. *Proc. Natl. Acad. Sci. USA.* 58:782–789.