REFERENCES AND NOTES

1. S. Porcelli *et al.*, *Nature* **341**, 447 (1989); S. Porcelli, C. T. Morita, M. B. Brenner, *ibid.* **360**, 593 (1992); E. M. Beckman *et al.*, *ibid.* **372**, 691 (1994).
2. P. A. Sieling *et al.*, *Science* **269**, 227 (1995); A. Bendelac, *ibid.*, p. 185.
3. S. A. Porcelli, *Adv. Immunol.* **59**, 1 (1995).
4. M. Sugita, unpublished observations.
5. P. J. Peters, J. J. Neefjes, V. Oorschot, H. L. Ploegh, H. J. Geuze, *Nature* **349**, 669 (1991); P. J. Peters *et al.*, *J. Exp. Med.* **182**, 325 (1995).
6. A. Tulp, D. Verwoerd, B. Dobberstein, H. L. Ploegh, J. Pieters, *Nature* **369**, 120 (1994).
7. F. Sanderson *et al.*, *Science* **266**, 1566 (1994).
8. H. W. Nijman *et al.*, *J. Exp. Med.* **182**, 163 (1995).
9. M. Sugita, P. J. Peters, S. A. Porcelli, unpublished observations.
10. A. Aruffo and B. Seed, *J. Immunol.* **143**, 1723 (1989); S. P. Balk, P. A. Bleicher, C. Terhorst, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 252 (1989).
11. I. V. Sandoval and O. Bakke, *Trends Cell Biol.* **4**, 292 (1994); F. Letourneur and R. D. Klausner, *Cell* **69**, 1143 (1992).
12. M. S. Marks *et al.*, *J. Cell Biol.* **131**, 351 (1995).
13. C. Harter and I. Mellman, *J. Cell Biol.* **117**, 311 (1992); J. S. Humphrey, P. J. Peters, L. C. Yuan, J. S. Bonifacino, *ibid.* **120**, 1123 (1993); S. Höning and W. Hunziker, *ibid.* **128**, 321 (1995); P. Voorhees *et al.*, *EMBO J.* **14**, 4961 (1995).
14. Complementary DNA for CD1b-TD was generated by polymerase chain reaction with the use of full-length CD1b cDNA (*1*) as a template DNA. The primers used were 5′-AGCGGATCCAGTGAACATGC-CTTCCAGGGG-3′ (plus strand, 5′ end of CD1b cDNA) and 5′-TCGGGATCCTAGCGCCTCATAT-ACCATAA-3′ (minus strand, 3′ end). The amplified fragment was sequenced and subcloned into pSRα-neo for transfection into HeLa cells. The cytoplasmic amino acid sequences of CD1b-FL and CD1b-TD are Met-Arg-Arg-Arg-Ser-Tyr-Gln-Asn-Ile-Pro and Met-Arg-Arg, respectively.
15. M. A. Williams and M. Fukuda, *J. Cell Biol.* **111**, 955 (1990).
16. R. A. Rogers, R. M. Jack, S. T. Furlong, *J. Cell Sci.* **106**, 485 (1993).
17. H. Ohno *et al.*, *Science* **269**, 1872 (1995).
18. P. G. Lerch, M. van de Rijn, P. Schrier, C. Terhorst, *Hum. Immunol.* **6**, 13 (1983).
19. V. Lotteau *et al.*, *Nature* **348**, 600 (1990); O. Bakke and B. Dobberstein, *Cell* **63**, 707 (1990).
20. M. Sugita and M. B. Brenner, *J. Biol. Chem.* **270**, 1443 (1995).
21. D. Olive, P. Dubreuil, C. Mawas, *Immunogenetics* **20**, 253 (1984).
22. C1R cells with high expression of CD1b were obtained by supertransfection of CD1b-positive C1R cells (*1*) with CD1b cDNA in a hygromycin-resistant gene containing expression vector p3-9, followed by selection with hygromycin B (200 μg/ml). The high-expressing cells were collected by fluorescence-activated cell sorting and used for immunoelectron microscopy analysis. Thin cryosectioning and immunogold labeling were carried out as described (*5, 8*), except for the use of the BCD1b3.1 antibody to CD1b [S. M. Behar, S. A. Porcelli, E. M. Beckman, M. B. Brenner, *J. Exp. Med.* **182**, 2007 (1995)] for detection of CD1b. Specificity of labelling with the antibody to CD1b was shown by the complete absence of labeling in mock-transfected (that is, $CD1b^-$) C1R cells. For each immunolabeling, at least 2000 cells were evaluated.
23. L. K. Denzin, N. F. Robbins, C. Carboy-Newcomb, P. Cresswell, *Immunity* **1**, 595 (1994).

24. We thank H. Ploegh, J. Trowsdale, M. Fukuda, P. Cresswell, D. Olive, C. Mawas, V. Hsu, and W. Brown for their gifts of reagents; L. Glimcher of Harvard Medical School for the gift of expression vector p3-9; Genetics Institute (Cambridge, MA) for GM-CSF; Schering-Plough Corp. (Kenilworth, NJ) for IL-4; and V. Hsu for critically reading the manuscript. Supported by grants from NIH.

31 January 1996; accepted 13 April 1996

Immunostimulatory DNA Sequences Necessary for Effective Intradermal Gene Immunization

Yukio Sato, Mark Roman, Helen Tighe, Delphine Lee, Maripat Corr, Minh-Duc Nguyen, Gregg J. Silverman, Martin Lotz, Dennis A. Carson, Eyal Raz*

Vaccination with naked DNA elicits cellular and humoral immune responses that have a T helper cell type 1 bias. However, plasmid vectors expressing large amounts of gene product do not necessarily induce immune responses to the encoded antigens. Instead, the immunogenicity of plasmid DNA (pDNA) requires short immunostimulatory DNA sequences (ISS) that contain a CpG dinucleotide in a particular base context. Human monocytes transfected with pDNA or double-stranded oligonucleotides containing the ISS, but not those transfected with ISS-deficient pDNA or oligonucleotides, transcribed large amounts of interferon-α, interferon-β, and interleukin-12. Although ISS are necessary for gene vaccination, they down-regulate gene expression and thus may interfere with gene replacement therapy by inducing proinflammatory cytokines.

Intramuscular (*1*) or intradermal (*2*) administration of pDNA expression vectors causes intracellular synthesis of the encoded proteins and induction of long-lasting cellular and humoral immune responses. Recently, we reported that mice injected or scratched intradermally with expression vectors encoding β-galactosidase (β-Gal) and containing a bacterial ampicillin resistance gene (*ampR*) produced a strong antibody response to β-Gal (*3*). However, subsequent experiments showed that mice injected intradermally with a similar expression vector containing the kanamycin resistance gene (*kanR*) instead of *ampR* generated a weak antibody response to β-Gal (Fig. 1 and Table 1). These results were unexpected, because we had always detected higher β-Gal activity in cells transfected with the *kanR*-based vector (pKCB) that encodes β-Gal, pKCB-Z (615.4 pg of β-Gal per well), than in cells transfected with the *ampR*-based vectors, pACB-Z and pACS-Z, encoding β-Gal (254.9 pg of β-Gal per well and 113.3 pg per well, respectively) (*4*).

To test the hypothesis that the *ampR* sequence may up-regulate the immune response to β-Gal in gene-vaccinated mice, we injected animals with pKCB-Z together with pDNA for either the *ampR* or *kanR* gene. The coadministration of pKCB-Z with vectors containing the *ampR* gene (pACB or pUC19) restored the antibody response to β-Gal to approximately the level induced by pACB-Z (Table 1). The immune stimulation was dose-related because coadministration of 100 μg of pUC19 induced a larger specific antibody response than coadministration of 5 μg of pUC19. In contrast, coadministration of pKCB-Z with the pKCB vector did not result in any significant enhancement of the antibody response to β-Gal (Table 1). The intradermal gene vaccination of mice with pKCB-Z did not induce a significant cytolytic T lymphocyte (CTL) response to β-Gal, as compared with the vigorous CTL response induced by pACB-Z (Fig. 2A). However, the coinjection of pKCB-Z with pACB or with pUC19 restored the CTL response to β-Gal to approximately the level observed in mice immunized with pACB-Z (Fig. 2A).

One of the main features of intradermal gene vaccination with naked pDNA is the induction of a T helper cell type 1 (T_H1) response to the gene product (*3, 5*). This response is characterized by the production of a distinctive cytokine profile [interleukin-2 (IL-2), tumor necrosis factor–β (TNF-β), and, mainly, interferon-γ (IFN-γ)] by antigen-stimulated CD4 T cells (*6*). The CD4 splenocytes from pACB-Z–immunized mice generated large amounts of IFN-γ and small amounts of IL-4 (Fig. 2, C and D, respectively), whereas cells from pKCB-Z–immunized mice produced only trace amounts of these cytokines. However, the production of IFN-γ could be restored in pKCB-Z–immunized mice by coinjection with the *ampR*-containing vectors, pACB and pUC19 (Fig. 2C).

Palindromic, single-stranded immunostimulatory DNA sequences (ISS) have been reported to induce production of IFN-α, IFN-β, and IFN-γ from mouse splenocytes and human peripheral lymphocytes and to enhance natural killer cell activity. These ISS include the following CpG-containing hexamers: 5′-GACGTC-3′, 5′-AGCGCT-3′, and 5′-AACGTT-3′ (*7*). Two repeats of 5′-AACGTT-3′ were in the *ampR*

Department of Medicine and The Sam and Rose Stein Institute for Research on Aging, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA.

* To whom correspondence should be addressed.

gene, whereas no ISS were identified within the *kanR* gene (Fig. 1). To test the hypothesis that the 5′-AACGTT-3′ ISS within the *ampR* gene facilitates the induction of CTL and T_H1 immune responses to β-Gal, we subcloned either one or two repeats of the ISS 5′-AACGTT-3′ to sites flanking the *kanR* gene in the pKCB-Z vector. The new vectors were designated pKISS-1-CB-Z and pKISS-2-CB-Z, respectively (Fig. 1). Gene vaccination with the modified pKISS vectors elicited strong humoral and cellular immune responses to β-Gal (Table 1 and Fig. 2). Notably, intradermal injection of the pKISS-2-CB-Z induced a stronger antibody response to β-Gal than did the pKISS-1-CB-Z.

The immunostimulatory effect of bacterial DNA was discovered by Tokunaga *et al.* (*8*). By synthesizing single-stranded oligonucleotides, corresponding to different regions in the *Mycobacterium bovis* genome, researchers identified specific single-strand-

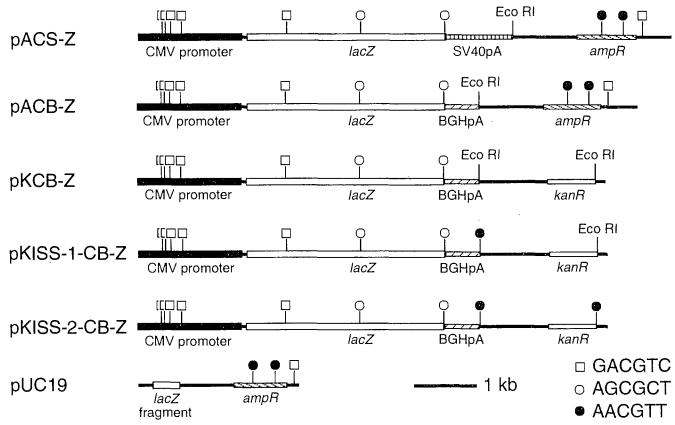

Fig. 1. Localization of the ISS on the pDNAs used in this study. The *kanR* gene does not contain any of the 5′-GACGTC-3′, 5′-AGCGCT-3′, and 5′-AACGTT-3′ palindromic ISS (*7*). The pACS and pACB vectors are pUC19-based plasmids (with *ampR*) that contain the cytomegalovirus (CMV) promoter-enhancer sequences [807 base pairs (bp)] along with the CMV immediate early intron (824 bp) (*2*). The pACS plasmid contains a 938-bp SV40 polyadenylate [poly(A)] (SV40pA) signal, whereas in the pACB plasmid, a 560-bp bovine growth hormone poly(A) signal (BGHpA) replaces the SV40pA signal (Bgl II–Eco RI). The pKCB vector was constructed by replacing the 1542-bp *ampR* gene (Bsp HI–Hind III) with a 1042-bp *kanR*-containing fragment (Bsp HI–Hind III). The *lacZ* expression vectors (pACB-Z and pKCB-Z) were constructed by inserting a 3283-bp *Escherichia coli lacZ*-containing fragment into the Pst I–Bam HI sites of the aforementioned corresponding vectors. The putative immunostimulatory double-stranded oligonucleotide (sense, 5′-AATTG**AACGTT**CGC-3′; antisense, 5′-AATTGCGAACGTTC-3′) flanked by Eco RI–compatible overhangs was ligated into a unique Eco RI site of pACB, 3′ to the BGHpA sequence. This resulted in the disruption of the Eco RI site and the creation of a new Psp1406I restriction site (AACGTT). The ISS-containing region was then subcloned (Bsp HI–Bam HI) into the pKCB and pKCB-Z vectors to create pKISS-1-CB and pKISS-1-CB-Z, respectively. The vectors pKISS-2-CB and pKISS-2-CB-Z were constructed by ligation of the same ISS oligonucleotide into pKISS-1-CB and pKISS-1-CB-Z at a different Eco RI site 5′ to the CMV promoter (disrupting this Eco RI site).

Table 1. Immunoglobulin G (IgG) to β-Gal in BALB/c mice co-immunized with various pDNAs (*22*). The antibody responses to β-Gal 8 weeks after immunization are shown (*23*). Intradermal injection of pKCB-Z and pUC19 into two separate sites (base of the tail and the nape, respectively) did not restore the immune response to β-Gal. Unless otherwise indicated, mice were immunized with 50 μg of pDNA. Results are the mean ± SE of eight mice for pKCB-Z and four mice for the other groups. In two other experiments, intradermal immunization of mice with pACS-Z or pACB-Z (eight mice per pDNA per experiment) resulted in similar responses.

Plasmid DNA	IgG to β-Gal (U/ml)
pKCB-Z	2018 ± 966*
pACS-Z	6391 ± 1401
pACB-Z	18052 ± 4842
pKCB-Z + pKCB (100 μg)	640 ± 260*
pKCB-Z + pACB (100 μg)	11248 ± 879
pKCB-Z + pUC19 (5 μg)	9505 ± 2522
pKCB-Z + pUC19 (100 μg)	59417 ± 17309
pKISS-1-CB-Z	34233 ± 5452
pKISS-2-CB-Z	47413 ± 11182

*Denotes $P < 0.05$ in comparison with the other groups by analysis of variance.

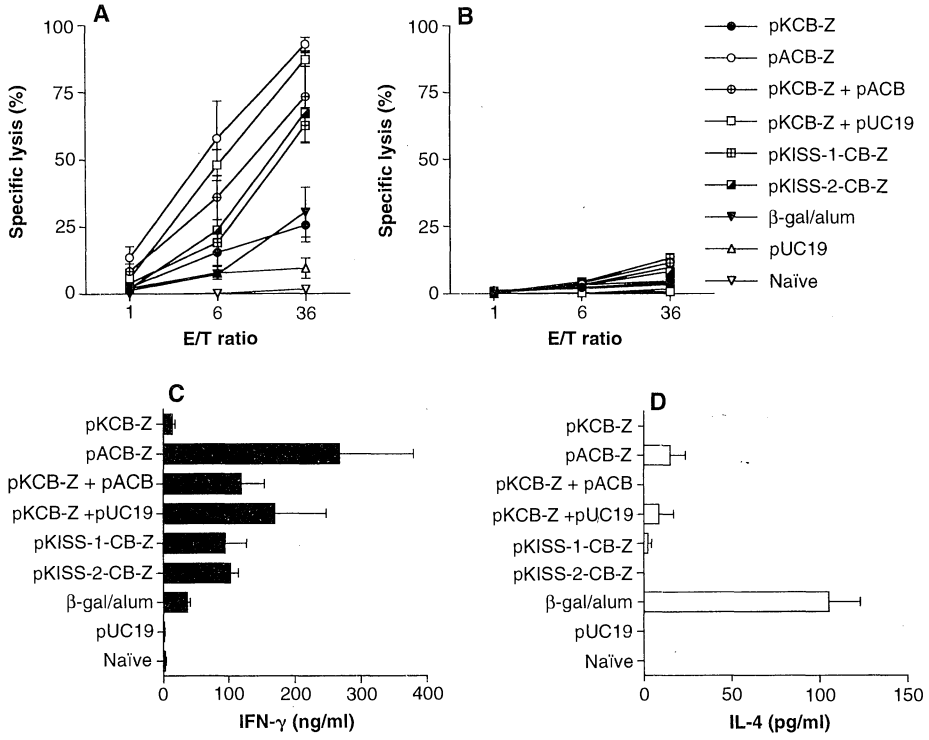

Fig. 2. Cellular immune responses to β-Gal in BALB/c mice immunized with various pDNAs (*22*). CTL and cytokine secretion assays were performed 12 weeks after initial immunization (*24*). (**A**) Restoration of the CTL response to β-Gal in mice by coinjection of *ampR*-based vectors (50 μg per injection for pKCB-Z, 100 μg for pKCB and pACB, and 5 μg for pUC19) or by injection of the modified pKCB vectors, pKISS-1-CB or pKISS-2-CB (50 μg per injection). The specific lysis of P815 target cells incubated briefly with β-Gal peptide was less than 10% in naïve, unimmunized mice and pUC19-immunized mice. (**B**) The specific lysis of P815 target cells incubated briefly with the influenza nucleoprotein peptide (*2, 24*) as an irrelevant peptide was less than 14% when the effector-to-target (E/T) ratio in all groups was 36:1. Secretion of IFN-γ (**C**) and IL-4 (**D**) by β-Gal–stimulated $CD4^+$ splenocytes. Coinjection of *ampR*-based vectors or injection of one of the modified pKCB vectors, pKISS-1-CB or pKISS-2-CB, enhanced IFN-γ production. $CD4^+$ splenocytes from gene-vaccinated mice secreted large amounts of IFN-γ and very small amounts of IL-4 compared with the $CD4^+$ splenocytes from mice vaccinated with β-Gal protein (Calbiochem, La Jolla, California) in alum, indicating a T_H1 response to β-Gal for the gene-vaccinated mice (*3*). Results are the mean ± SE of four mice per group.

ed oligonucleotides that activated adherent splenocytes and enhanced natural killer cell activity in vitro (9). Recently, Krieg *et al.* studied the effects of single-stranded oligonucleotides with CpG motifs on murine B lymphocyte activation (*10*). They found that cytosine methylation or the elimination of the CpG from the oligonucleotide abolished the lymphocyte stimulatory effect. The activation capability was attributed to a series of CpG-containing motifs that generally follow the formula 5′-Pur Pur CG Pyr Pyr-3′. CpG-enriched oligonucleotides induced not only B cell proliferation, but also the secretion of IL-6 and IFN-γ (*11*).

To analyze the cytokine profile induced by the *kanR*- and by the *ampR*-based vectors, we transfected in vitro fresh human monocytes with a panel of pDNAs, ISS oligonucleotide, and ISS-deficient oligonucleotide (*12, 13*) and then assessed by reverse transcription–polymerase chain reaction (RT-PCR) the expression of the T_H1-associated cytokine mRNAs of IFN-α, IFN-β, and the p40 subunit of IL-12. Transfection with pUC19, pACB, pKISS-1-CB, and double-stranded ISS digonucleotide, but not with pKCB or ISS-deficient oligonucleotide, enhanced within 3 hours mRNA amounts for all three cytokines (*12, 13*). IFN-α plays a role in the differentiation of naïve T cells toward a T_H1 phenotype, antagonizes T_H2 cells (*14*), inhibits IgE synthesis, promotes IgG2a production (*15, 16*), and induces a T_H1 phenotype of allergen-specific T cell clones (*17*). IL-12 promotes IFN-γ production by T cells (*18*) and favors maturation of T_H1 cells (*19*). Recently, Halpern *et al.* showed that the stimulation of IFN-γ synthesis by bacterial DNA is mediated by IL-12 and TNF-α (*20*). Therefore, keratinocytes and dermal antigen-presenting cells (APCs) transfected with ISS-containing pDNA could produce IFN-α and IL-12, which would then induce a T_H1 immune response against the pDNA-encoded protein.

Our findings indicate that immunogenic pDNA may be divided conceptually into two distinct units: a transcription unit that directs antigen synthesis and an adjuvant unit in the plasmid backbone that elicits the production of type-1 IFN and IL-12 in the transfected skin keratinocytes and APCs. For this reason, manipulation of the transcription unit within the pDNA to yield higher levels of antigen expression does not necessarily produce a stronger immune response. Both the localization and the precise sequence of the ISS within the plasmid backbone are also important for DNA vaccination. Thus, the potential ISS, 5′-GACGTC-3′, and 5′-AGCGCT-3′ in the pKCB-Z transcription unit did not have sufficient adjuvant activity in vivo (Fig. 1). In contrast, the addition of one or two repeats of the 5′-AACGTT-3′ sequence to the noncoding region of the pKCB-Z backbone enhanced the immune response to β-Gal in a "gene dosage"–related fashion (compare pKISS-1-CB-Z with pKISS-2-CB-Z in Table 1).

Although ISS enhance CTL and T_H1 immune responses after intradermal gene vaccination, they may interfere with gene expression by stimulating IFN-α production. Indeed, pKISS-1-CB-Z, pKISS-2-CB-Z, and *ampR*-based vectors always expressed smaller amounts of β-Gal in the transfected cells than did pKCB-Z, and the addition of neutralizing antibodies to IFN-α doubled β-Gal expression in the pACB-Z–transfected MG-63 cell line, whereas the addition of IFN-α (50 pg/ml) diminished β-Gal expression in pKCB-Z–transfected cells by 40% (*12*). Thus, the presence of ISS may interfere with gene replacement therapy by inducing the synthesis of IFN-α and proinflammatory cytokines. The IFN-α produced by transfected somatic or stem cells may directly impede mRNA and protein synthesis (*21*). ISS-containing plasmids transfected into bone marrow stem cells may activate adjacent macrophages and T lymphocytes, with resultant impairment of stem cell reconstitution in vivo. A DNA adjuvant effect is desirable for gene immunization, but unnecessary and perhaps harmful for gene replacement. Vectors for somatic or stem cell gene replacement therapy should be designed to lack these ISS, whereas pDNA for gene vaccination should be engineered to have multiple repeats of the ISS.

REFERENCES AND NOTES

1. E. F. Fynan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 11478 (1993); J. B. Ulmer *et al.*, *Science* **259**, 1745 (1993); B. Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4156 (1993).
2. E. Raz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9519 (1994).
3. E. Raz *et al.*, *ibid.* **93**, 5141 (1996).
4. MG-63 cells (American Type Culture Collection) were seeded at 2×10^5 cells per well in a 24-well plate the day before transfection with 2 μg of pDNA and DOTAP (Boehringer Mannheim). The β-Gal assays were performed as described [J. Felgner *et al.*, *J. Biol. Chem.* **269**, 2550 (1994)]. Data are the average of duplicates and represent one of three similar experiments. The β-Gal amounts from MG-63 cells transfected with pKISS-1-CB-Z and pKISS-2-CB-Z were 400.0 pg per well and 360.6 pg per well, respectively.
5. E. Manickan, R. J. D. Rouse, Z. Yu, W. S. Wire, B. T. Rouse, *J. Immunol.* **155**, 259 (1995); Z. Xiang and H. C. J. Ertl, *Immunity* **2**, 129 (1995).
6. T. R. Mosmann and R. L. Coffman, *Annu. Rev. Immunol.* **7**, 145 (1989).
7. S. Yamamoto *et al.*, *J. Immunol.* **148**, 4072 (1992); T. Yamamoto *et al.*, *Jpn. J. Cancer Res.* **85**, 775 (1994); T. Yamamoto, S. Yamamoto, T. Kataoka, T. Tokunaga, *Microbiol. Immunol.* **38**, 831 (1994).
8. T. Tokunaga *et al.*, *J. Natl. Cancer Inst.* **72**, 955 (1984).
9. T. Tokunaga *et al.*, *Microbiol. Immunol.* **36**, 55 (1992); Y. Kimura *et al.*, *J. Biochem.* **116**, 991 (1994).
10. A. M. Krieg *et al.*, *Nature* **374**, 546 (1995).
11. A.-K. Yi, J. H. Chace, J. S. Cowdery, A. M. Krieg, *J. Immunol.* **156**, 558 (1996).
12. Y. Sato *et al.*, unpublished data.
13. The sequences of the annealed, blunt-end, double-stranded oligonucleotides used for transfection are as follows: ISS oligonucleotide, 5′-TCATTG-GAA**AACGTT**CTT**CG**GGG**CG**-3′, from the *ampR* gene in the pUC19 sequence (nucleotides 2288 to 2312); and ISS-deficient oligonucleotide, 5′-TCATT-GGAA**AAGGTT**CTT**GG**GGG**GG**-3′. Bold nucleotides indicate the ISS.
14. V. Brinkmann, T. Geiger, S. Alkan, C. H. Heusser, *J. Exp. Med.* **178**, 1655 (1993).
15. F. D. Finkelman *et al.*, *ibid.* **174**, 1179 (1991).
16. P. Parronchi *et al.*, *J. Immunol.* **149**, 2977 (1992).
17. R. Manetti *et al.*, *Eur. J. Immunol.* **25**, 2656 (1995).
18. G. Trinchieri, *Annu. Rev. Immunol.* **13**, 251 (1995).
19. C.-S. Hsieh *et al.*, *Science* **260**, 547 (1993).
20. M. D. Halpern, R. J. Kurlander, D. S. Pisetsky, *Cell. Immunol.* **167**, 72 (1996).
21. J.-C. Wang, H. D. Lang, P. Liao, A. Wong, *Am. J. Hematol.* **40**, 81 (1992).
22. We prepared pDNA with a Qiagen Maxi Kit (Qiagen, Chatsworth, CA). Endotoxin was removed by Triton X-114 (Sigma, St Louis, MO) extractions [Y. Aida and M. J. Pabst, *J. Immunol. Methods* **132**, 191 (1990)]. Endotoxin amounts were determined with the Pyrotell^R limulus amebocyte lysate (LAL) assay (Association Cape Cod, Woods Hole, MA) and were less than 5 ng per milligram of DNA. Female BALB/c mice were intradermally injected at the base of the tail three times, 10 days apart, with the various pDNAs dissolved in normal saline. Mice were immunized with β-Gal protein in alum as previously described (*3*). All animal studies were approved by the University of California, San Diego, animal welfare committee.
23. The enzyme-linked immunosorbent assay (ELISA) for antibody to β-Gal was described previously (*3*). To quantitate the relative amount of antibody, we compared titration curves for individual sera with a standard curve on each plate using DeltaSOFT II v. 3.66 software (BioMetallics, Princeton, NJ). Additional experiments showed that the effects of the ISS were not attributable to polyclonal activation (Y. Sato *et al.*, data not shown).
24. The CTL assay was described previously (*2*). Splenocytes from unimmunized mice or mice immunized with either pDNA or β-Gal protein were isolated 12 weeks after the first immunization and cultured with irradiated syngeneic splenocytes that had been incubated for 5 days with the β-Gal–derived, class I H-2L^d–restricted nanopeptide (Thr Pro His Pro Ala Arg Ile Gly Leu) (4 μg/ml) [M. A. Gavin, M. J. Gilbert, S. R. Riddell, P. D. Greenberg, M. J. Bevan, *J. Immunol.* **151**, 3971 (1993)]. After 5 days, the restimulated cells were harvested and separated from dead cells on a Lympholyte M gradient (Accurate Chemicals, Westbury, NY). In 96-well round-bottom plates, 10,000 P815 target cells incubated with either the β-Gal–derived peptide or a control class I H-2K^d–restricted peptide, derived from the influenza nucleoprotein (Thr-Tyr-Gln-Arg-Thr-Arg-Ala-Leu-Val), were incubated with the restimulated T cells in graded effector-to-target ratios for 4 hours. The specific lysis was assessed by measuring lactate dehydrogenase release into supernatants with the Cyto Tox 96 assay kit (Promega, Madison, WI). IFN-γ and IL-4 secretion by β-Gal–activated CD4$^+$ splenocytes were assessed as previously described (*3*) except that commercial ELISAs were used (Endogen, Cambridge, MA).

25. We thank J. A. Dudler, Y. Geng, and J. Quach for providing the pACB vector, IL-12 p40 primer, and fresh human monocytes. We also thank P. Charos, A. Ronaghy, and S. Malek for their technical assistance and N. Noon for editorial assistance. Supported in part by grants AI37305, AR41897, and AI36214 from NIH. Y.S. was supported in part by the Japan Rheumatism Foundation. M.R. was supported by an NIH training grant (AI07384). H.T. was supported in part by an award from the CaPCURE Foundation.

29 February 1996; accepted 10 May 1996