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A Targeted DNA Vaccine Augments the Natural Immune Response to Self TNF-α and Suppresses Ongoing Adjuvant Arthritis

Gizi Wildbaum,* Sawsan Youssef,* and Nathan Karin2*†

Depending on the mode of immunization, a single administration of CFA may result in the development of a local inflammatory process or chronic poly adjuvant-induced arthritis (AA). Administration of naked DNA encoding TNF-α results in the generation of immunological memory to its gene product. Upon induction of AA, this memory effectively inhibited the development of disease. Self-specific Abs developed in DNA-vaccinated animals were neutralizing in vitro and could adoptively transfer the beneficial effect of the vaccine. Administration of CFA to induce a local delayed-type hypersensitivity response rather than AA did not lead to an elicited production of Abs to the gene product of the above vaccine. Thus, elicitation of protective immunity is dependent on the development of an autoimmune condition. Most importantly, the administration of the TNF-α DNA construct after the onset of disease led to a rapid, long-lasting remission. This suggests a highly effective way by which a DNA vaccine encoding an autologous proinflammatory cytokine can be used to reprogram the immune system to generate protective immunity to its own potentially harmful activities. The Journal of Immunology, 2000, 165: 5860–5866.

Rheumatoid arthritis is an inflammatory disorder characterized by infiltration of leukocytes into synovial tissue and synovial fluid of joints (1). Depending on the mode of immunization, a single administration of CFA may result in the development of a local inflammatory process or chronic poly adjuvant-induced arthritis (AA) that histologically and clinically resembles human rheumatoid arthritis (2). In both diseases, proinflammatory cytokines and chemokines are believed to play a pivotal role in the attraction of leukocytes to the site of inflammation and in the initiation and progression of the inflammatory process. The role of proinflammatory cytokines, particularly TNF-α and IL-1, in disease manifestation has been intensively studied and explored in experimental models that have been expanded in clinical trials (3–8) (for general review, see also Ref. 9). A major disadvantage in treating long-lasting autoimmune diseases with neutralizing Abs (or soluble receptor immunotherapy) to proinflammatory mediators lies in their short half-life time, which requires their repeated administration. We have recently explored a modification of naked DNA vaccination as a potential way to circumvent this drawback (10–12).

We have cloned cDNA encoding various proinflammatory cytokines/chemokines including TNF-α, monocyte chemotactant protein-1 (MCP-1), and macrophage-inflammatory protein-1α into a mammalian vector with a strong viral promoter (CMV) and a recurring immunostimulatory sequence (13, 14). Upon repeated administrations, tolerance to the product of each gene of interest was broken and immunological memory established. Following the initiation of experimental autoimmune encephalomyelitis (EAE), a T cell-mediated autoimmune disease of the CNS that serves as an experimental model for multiple sclerosis, this memory was turned on to provide protective immunity. Thus, administration of either TNF-α, macrophage-inflammatory protein-1α, or MCP-1 DNA vaccines led to a high state of EAE resistance in Lewis rats that could be adoptively transferred by the neutralizing Abs generated in response to each gene product. Surprisingly, elicitation of these Abs was found to be dependent on the development of the autoimmune condition and regulated by the immune system in accordance with disease progression (10, 11). This could provide the immune system of a patient with an autoimmune disease a powerful tool with which to restrain its own harmful activities. A major disadvantage of exploring the therapeutic potential of cytokine/chemokine-based DNA vaccines using the EAE model in the Lewis rat is that both the active and the transferred form of disease manifest only a transient disease. This model is thus impractical for the exploration of the effect of such therapy in chronic or recurrent disease states, particularly when DNA vaccination was planned for administration after the onset of disease. Under our working conditions, Lewis rats manifest a long-term AA that includes an acute phase and a long-lasting (more than 100 days) chronic phase of disease. The current study explores, for the first time, TNF-α-based naked DNA vaccination in AA and reveals its therapeutic potential.

Materials and Methods

Rats

Female Lewis rats, approximately 6 wk old, were purchased from Harlan (Jerusalem, Israel) and maintained under clean conditions in our animal facility.

Immunizations and active disease induction

Rats were immunized s.c. in the base tail with 0.1 ml of CFA (IFA supplemented with 10 mg/ml heat-killed Mycobacterium tuberculosis H37Ra

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in oil; Difco Laboratories, Detroit, MI). Rats were then monitored for clinical signs daily by an observer blind to the treatment protocol, as we described elsewhere (15). Severity of the disease was quantified subjectively by grading a scale on a scale of 0–4 to indicate the severity of peripheral joint swelling and erythema: 0 = no signs of disease; 1 = disease evident in a small number of distal joints of the limb; 2 = disease evident in all of distal joints of the limb; 3 = disease evident in all the limb; 4 = severe disease evident in all the limb. The arthritic clinical score was determined as the sum of the scores of all four limbs from each animal (0–16). An observer blind to the experimental protocol quantified the degree of arthritis, indicated by swelling, by measuring front and hind limb circumference using a caliper (Lange Skinfold Caliper; Cambridge Scientific Industries, Cambridge, MA).

**DNA vaccination**

The sequenced PCR product of rat TNF-α (10) was transferred into a pcDNA3 vector (Invitrogen, San Diego, CA). Large-scale preparation of plasmid DNA was conducted using Mega prep (Qiagen, Chatsworth, CA). Cardiotoxin (Sigma, St. Louis, MO) was injected into the tibialis anterior muscle of 6- to 8- wk-old female Lewis rats (10 μM per leg). In addition, cDNA encoding rat β-actin (the natural cytoplasmic soluble form of β-actin) has been obtained using specific oligonucleotide primers (sense, 5'-ATGGATGACGATATCGCTGCGCTC-3'; antisense, 5'-CTACCCGGC CAGCCGACG-3'). Following cloning and sequence verification, the above cDNA was ligated into the pcDNA3 vector to be used as a control DNA vaccine. Following the toxin injection, rats were injected with 100 μg of the pcDNA3 vector pcDNA3 vector with inserts encoding either TNF-α or soluble β-actin. Four to five days after the first immunization, one rat from each group was sacrificed. RT-PCR was applied on tibialis anterior muscle samples. At that time, muscle samples form the rat subjected to DNA vaccine encoding TNF-α exhibited an elevated transcription of TNF-α, but not of soluble β-actin, and vice versa. Thus, the relevant insert of each inserted gene is transcribed in the injected muscle. Next, naked DNA vaccine was administered four times with intervals of 6–7 days between each injection.

**Evaluation of TNF-α-specific Ab titer in sera of DNA-vaccinated rats**

A direct ELISA assay was used to determine the anti-TNF-α Ab titer in DNA-vaccinated rats. ELISA plates (Nunc, Roskilde, Denmark) were coated with 50 ng/well commercially available rat rTNF-α (Genzyme, Cambridge, MA). Sera from DNA-vaccinated rats were added in serial dilutions from 25 to 2^30 to wells that were, or were not, coated previously with rTNF-α. Calculation of each titer was done by comparing the OD measured in wells coated with TNF-α with those not coated with this recombinant cytokine. Goat anti-rat alkaline phosphatase-conjugated IgG Abs (Sigma) were used as a labeled Ab. p-Nitrophenyl phosphate (Sigma) was used as a soluble alkaline phosphatase substrate. Results of triplicates were calculated as log_{10} Ab titer ≥ SE.

**Purification of Abs**

Sera obtained from TNF-α DNA-vaccinated rats were subjected to two steps of purification before being tested for their in vitro neutralizing characteristics and their ability to affect the course of AA. At first, a High-Trap Protein G column (Pharmacia, Piscataway, NJ) was used (according to the manufacturer’s protocol) to purify the IgG fraction. Then TNF-α-specific Abs were purified using a TNF-α-CNBr-activated Sepharose column, as follows: commercially available rTNF-α (Genzyme, Cambridge, MA) was bound to a CNBr-activated Sepharose column, according to the manufacturer’s instructions (Pharmacia Biotech, Uppsala, Sweden; catalog number 17-0820-01). The IgG fraction was then loaded on the column and eluted by acidic buffer (glycine pH 2.5). Isotype determination (ELISA) revealed that TNF-α-specific Abs obtained from DNA-vaccinated rats are mostly of the IgG2a isotype (data not shown).

**Determination of the neutralizing activity of TNF-α-specific Abs**

Determination of the neutralizing activity of TNF-α-specific Abs was done as described (16), with the modification of using the U937 monocyte cell line (CRL-1593.2; American Type Culture Collection, Manassas, VA), at a concentration of 4 × 10^5 cells/well, as a target cell for the assay (10).

**Histopathology**

Joints were removed at various time points after disease induction, fixed with 10% buffered formalin, decalcified in 5% EDTA in buffered formalin, embedded in paraffin, and sectioned along the midline through the metatarsal region (17). Sections were stained with hematoxylin and eosin and analyzed by a histopathologist blind to the experimental procedure. Evaluation was based upon the level of inflammatory mononuclear cell infiltrate in the synovial membrane, thickness of the synovial lining, joint space narrowing, and periosteal new bone formation. Clinical score was determined as follows: 0 = no evidence of disease; 1 = mild lymphocytic infiltrate; 2 = widespread mononuclear inflammation and thickening of the synovial lining; and 3 = severe bone destruction, new bone formation, and destruction of the synovial lining (17).

**Statistical analysis**

Significance of differences was examined using Student’s t test. A value of p < 0.05 was considered significant. Mann-Whitney sum of ranks test was used to evaluate significance of differences in mean of maximal clinical score, with p < 0.05 considered significant.

**Results**

**Prevention of AA using TNF-α naked DNA vaccine**

The cloned PCR product of TNF-α was ligated into a pcDNA3 mammalian expression vector and used for naked DNA vaccination. Lewis rats were exposed to four weekly administrations of this construct. Control rats were injected with either a β-actin construct, pcDNA3 vector alone, or PBS. Three weeks after the last immunization, all rats were immunized with CFA to induce AA. Under our working conditions, AA manifests a long-lasting form of disease that includes an acute phase, peaking at about day 20, and a chronic phase that persists for more than 100 days (Fig. 1). All control rats treated with either PBS, pcDNA3 alone, or β-actin pcDNA3 (12 per group) developed a severe form of disease, with a maximal clinical score (day 20) of 13.5 ± 1.8, 13 ± 1.52, and 13 ± 1.52, respectively (Fig. 1). In contrast, rats subjected to the administration of the TNF-α construct developed a significantly reduced form of disease (mean maximal score of 6.7 ± 1.1, p < 0.001 compared with each of the control groups). A significantly reduced form of disease was also observed in these animals during the chronic phase of disease (day 45, 1.7 ± 0.7 vs 7.2 ± 0.77, 6.5 ± 1, and 6.7 ± 1.2, p < 0.001; day 90, 1 ± 0.7 vs 3.3 ± 0.8, 3.3 ± 0.86, and 3.5 ± 0.7, p < 0.001). Clinical scoring has also been verified by measuring the changes in the degree of paw swelling, once again by an observer blind to the experimental procedure. At all times (days 20, 45, and 90), TNF-α DNA-vaccinated

**FIGURE 1.** Prevention of AA by a TNF-α-targeted DNA vaccine. Groups of 12 Lewis rats were exposed to four weekly administrations of TNF-α DNA vaccine. Control rats were injected with either a β-actin construct, pcDNA3 vector alone, or PBS. Three weeks after the last immunization, all rats were immunized with CFA to induce active AA. AA was scored (daily during the acute phase of disease, weekly during its chronic phase) by an observer blind to the experimental procedure. Leg swelling was also determined (data not shown). On day 30, four representative rats per group were sacrificed and joints were removed for histological analysis. Clinical score (A) and the differences in leg swelling (B-D) are shown as mean of 12 rats (days 10–30), or 8 rats (day 31 onward) ± SE.
rats exhibited a marked reduction in Δ paw swelling compared with each of the control groups (p < 0.001, data not shown). Additionally, representative joint sections from all experimental groups (four animals per group) were obtained on day 30 and screened for histological inflammatory mononuclear cell infiltrate in the synovial membrane, thickness of the synovial lining, joint space narrowing, and periostial new bone formation. Sections obtained from TNF-α DNA-vaccinated rats displayed a marked reduction in each of the above parameters as compared with control and pcDNA3-treated AA rats (mean histological score of 12 sections from four animals, 0.5 ± 0.2 in TNF-α DNA-vaccinated rats compared with 2.8 ± 0.2, 2.66 ± 0.4, and 3 ± 0 in pcDNA3-, β-actin-, or PBS-treated rats, p < 0.001, respectively). Thus, TNF-α-based naked DNA vaccination can serve as a powerful tool to prevent AA.

Self-specific Abs developed in DNA-vaccinated rats are neutralizing in vitro and capable of transferring the protective effect of each vaccine

DNA vaccination can potentially elicit both cellular and humoral responses against products of a given construct (18–22). To assess the contribution of the humoral response to the tolerant state, DNA-vaccinated AA rats were followed for the production of Abs to TNF-α. Then Abs were evaluated for their ability to neutralize TNF-α (in vitro) and to interfere in the development of AA in an adoptive transfer experiment. Thus, Lewis rats were subjected to administration of PBS, pcDNA3 alone, β-actin construct, or TNF-α naked DNA. Three weeks later, these rats were separated to subgroups that were immunized with CFA in one of two different ways: 1) footpad injection to induce a local delayed-type hypersensitivity (DTH) response, or 2) tail-base administration to induce polyarthritis. At the peak of the acute phase of disease, the appearance of anti-TNF-α Ab in the serum was determined. Rats developing polyarthritis develop Ab to TNF-α (Fig. 2, A and B), but not to β-actin (data not shown), even without DNA vaccination. Interestingly, this titer differed significantly not only from the one in naive rats, but also from that in rats immunized to manifest a local DTH response (Fig. 2, on day 20 log₂ Ab titer of 13 ± 0.55 in AA rats vs 9 ± 0.33 and 8 ± 0.47 in rats with developing a local DTH response and naive rats, respectively, p < 0.05 for the comparison of AA rats with each of the control groups). However, this increase in titer was insufficient to prevent the development and progression of inflammation in AA (Fig. 1). In TNF-α naked DNA-vaccinated rats, TNF-α-specific Ab titer was elicited following AA induction, and to a much lesser extent following the injection of CFA to induce a DTH response, Fig. 2A (log₂ Ab titer of 25 ± 1.2 vs 16 ± 0.8, p < 0.001). The above elicited Ab titer continued to persist during the chronic phase of disease (Fig. 2C), as did the clinical effect of these vaccines (Fig. 1). To determine the specificity of anti-TNF-α Abs, even at the lower dilutions, rTNF-α was added at various concentrations to sera from rats with developing active AA (day 20, log₂ Ab titer of 13 ± 0.3). Then the Ab titer was reassessed. It appears that as much as 0.1 ng/ml of rTNF-α was required to reduce this titer back to the one observed in control rats immunized with CFA to induce a local inflammatory process (log₂ Ab titer of 9 ± 0.3).

Abs were purified (IgG fraction, protein G purification) and evaluated for their competence to neutralize the activity of TNF-α (in vitro) and transfer AA resistance. IgG from TNF-α DNA-vaccinated rats abolished the cytotoxic activity of TNF-α on U937 cells (Fig. 3). (Neutral red uptake as OD at 570 nm was 0.18 ± 0.007 vs 0.08 ± 0.008, 0.075 ± 0.004, and 0.05 ± 0.008 in the presence of 100 μg/well IgG from TNF-α DNA-vaccinated rats, normal IgG, IgG from pcDNA3-immunized rats, or PBS and 100 pg/ml TNF-α, with backgrounds of 0.17, 0.19, 0.19, and 0.21, respectively, p < 0.001 for the comparison of the first group with each of the control groups.) Thus, neutralizing Abs are produced in naked DNA-vaccinated rats. Purified Abs were then evaluated for their competence to provide protection from severe ongoing AA (Fig. 4). Beginning on day 16 after initiation of AA, rats were

![FIGURE 2. Specific DNA vaccination leads to elevation of specific Ab titers especially in AA rats. Lewis rats were subjected to the administration of TNF-α DNA construct, as described in legend to Fig. 1. Control rats were injected with either a β-actin construct, pcDNA3 alone, or PBS. Three weeks later, these rats were separated to subgroups that were administered with CFA in two different ways: 1) Footpad injection to induce a local DTH response. 2) Tail-base administration to induce polyarthritis. At various time points, titers of anti-TNF-α in serum were determined by ELISA. A, Comparative analysis of Ab titer to TNF-α developed in each group on day 20. B, Follows the kinetics of Abs to TNF-α generated after following administration of CFA to induce a local DTH response, or chronic AA in rats not exposed to DNA vaccine. C, Follows the kinetics of self-specific Abs to TNF-α generated after administration of CFA to induce a local DTH response, or chronic AA in DNA-vaccinated rats. Results are the mean of three different serum samples ± SE.](http://www.jimmunol.org/)

![FIGURE 3. TNF-α-specific Abs produced by DNA vaccination are neutralizing. TNF-α-specific Abs (100 μg/well) purified from sera of rats previously vaccinated with TNF-α naked DNA vaccine and subjected to active induction of AA were evaluated for their competence to inhibit neutral red uptake by U937 cells (4 × 10⁵/well in a total volume of 100 μl). IgG purified from normal rat serum (100 μg/well), IgG fraction from pcDNA3-vaccinated AA rats (100 μg/well), or an equal volume of PBS were used as controls. Results of triplicates are shown as mean OD at 570 nm ± SD.)](http://www.jimmunol.org/)

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challenged (i.v.) with 100 μg of each of these TNF-α-specific Abs. Control rats were injected with either PBS, IgG from naive rats, or from AA rats previously administered with pcDNA3 alone. Repeated administration of TNF-α-specific Abs from DNA-vaccinated rats led to a marked reduction in disease severity as compared with all control groups (day 20, mean maximal score of 2.25 ± 0.7 compared with 9.5 ± 1.6, 10 ± 1.4, 10 ± 0.7, and 10.5 ± 1.4 in AA rats treated with either PBS, IgG from pcDNA3-vaccinated rats, or IgG from naive rats, p < 0.001 for the comparison with each of the control groups). Clinical scoring has also been quantitated by measuring limb swelling and verified histologically (not shown). Taken together, these results may explain, in part, the effect of TNF-α DNA vaccination on disease manifestation. Five to seven days after the last administration of neutralizing Abs to TNF-α, disease severity regained the level of Ab-untreated AA rats (not shown). This transient effect of Ab administration further emphasizes the advantageous of naked DNA vaccination over neutralizing Ab therapy.

Treatment of an established disease by TNF-α-encoding DNA vaccine

From the practical perspective, we found it highly important to evaluate the competence of the TNF-α naked DNA to interfere with the progression of an ongoing disease when the vaccine is administered after the onset of disease. Thus, Lewis rats were immunized with CFA to induce active AA and divided into four random groups of 12 rats each. One day after the onset of disease (day 11) and on days 13 and 15, each group was subjected to administration of either PBS, pcDNA3 alone, a β-actin construct, or the TNF-α construct (300 μg per rat). While all control and pcDNA3-treated rats continued to develop severe AA, those exposed to the TNF-α DNA vaccine exhibited a markedly reduced disease severity (Fig. 5, day 20, mean maximal score of 12 ± 0.9 and 10.2 ± 0.62 and 10.5 ± 1.3 in rats treated with either PBS, β-actin construct, or pcDNA3 alone vs 6.2 ± 0.76, p < 0.001 for the comparison of TNF-α DNA-vaccinated rats to each control group; day 25, 11.5 ± 1, 11.5 ± 1, and 11.2 ± 0.9 vs 5.3 ± 0.7, respectively, p < 0.001). The marked reduction in disease severity persisted during the chronic phase of disease (day 60, 4.8 ± 0.8, 5.2 ± 0.8, and 5.5 ± 0.66 vs 1.5 ± 0.7, respectively, p < 0.001). Clinical scores were confirmed histologically (Fig. 6). Thus, 30 and 60 days after disease induction, representative joint sections (four rats per group) were obtained from rats that were treated with the TNF-α construct, pcDNA3 alone, or PBS, and evaluated for histological analysis of synovitis, cartilage loss, and bone erosion, compared with sections from naive rats. A and B show naive joints taken with age matching to the experiment rats (day 30); C and D, naive joints that were taken with age matching to the experiment rats (day 60). E and F show arthritic joints taken 30 days after disease induction; G and H, arthritic joints taken 90 days after disease induction. I and J show pcDNA3-treated joints taken 30 days after disease induction; K and L show pcDNA3-treated joints taken 60 days after disease induction. M and N show TNF-α-treated joints taken 30 days after disease induction; O and P show TNF-α-treated joints taken 60 days after disease induction. The arrowheads point to the synovial lining (b = bones, S = synovial membrane). The above representative sections clearly show that AA rats treated with the TNF-α DNA construct displayed a marked reduction in each of the above parameters as compared with untreated and pcDNA3-treated AA rats. The beneficial effect of the treatment was long lasting and covered not only the acute phase (day 30), but also the chronic phase of disease (day 60). During the acute phase of disease, sections from PBS- and pcDNA3-treated control rats displayed a massive inflammatory mononuclear cell infiltrate in the synovial membrane, an increase in thickness of the synovial lining, narrowing of the joint space, and notable periosteal new bone formation (Fig. 6, E and F, and I and J). At this stage of disease, sections from TNF-α naked DNA-treated rats displayed a substantial reduction in synovial leukocyte infiltration, synovitis, cartilage loss, and bone erosion (Fig. 6, M and N). During the chronic phase of disease, the intensity of the synovial leukocyte infiltration regressed, while cartilage loss, bone erosion, and periosteal new bone formation increased (Fig. 6, G and H, and K and L). Interestingly, and most importantly, during the chronic phase of disease (day 60), massive cartilage loss, bone erosion, and periosteal new bone formation that characterized control and pcDNA3-treated rats were entirely absent in joint sections of rats treated with TNF-α naked DNA vaccine after the induction of AA (Fig. 6, G and H). Thus, TNF-α naked DNA vaccination can serve as a highly effective treatment for ongoing arthritis.

In an additional experiment, Lewis rats were immunized with CFA to induce active AA. Seven days after the onset of disease (day 18), these rats were separated into four equal groups of six rats each and repeatedly (day 18, 20, 21) with either PBS, pcDNA3 alone, β-actin construct, or our TNF-α construct, as described above. Surprisingly, in all (6/6) TNF-α DNA-vaccinated rats, the arthritic score was markedly reduced within 5–6 days after the DNA vaccine was administered (Fig. 5B, mean maximal score 2 ± 0 vs 14.1 ± 0.8, p < 0.001). These results are unexpected, since the process of expressing a transgene and generation of an immune response against its gene product should have taken much longer time. A partial explanation for these unexpected, yet highly interesting observations could be obtained from Fig. 5C. Six days after administration of each DNA vaccine, sera were obtained from rats...
treated with either β-actin- or TNF-α-encoding DNA and evaluated for TNF-α- vs β-actin-specific Ab titer, compared with non-treated (PBS) AA rats. Similar to the results presented in Fig. 3, even without being subjected to TNF-α-encoding DNA vaccine, AA rats did mount a significant \( p < 0.05 \) Ab titer to TNF-α, but not to β-actin. This titer rapidly accelerated following the administration of TNF-α, but not β-actin-encoding DNA vaccine (Fig. 5C, \( p < 0.0001 \)). Interestingly, β-actin-encoding DNA vaccine did not elicit anti-self Ab production within 6 days (Fig. 5C). Taken together, these results suggest that TNF-α DNA vaccines interfere and amplify a preexisting response. This can provide a partial explanation to the fast effect of this vaccine, as observed in Fig. 5, A and B.

Discussion

The current study uses naked DNA vaccination to generate protective immunity against an autologous soluble cytokine associated with the establishment and the development of the autoimmune process in rheumatoid arthritis. We demonstrate that lymphocytes capable of mounting immunity to a proinflammatory cytokine (TNF-α) are activated during the course of an autoimmune condition (polyarthritis). The response they mount is, however, not sufficient to prevent the development and progression of the autoimmune disease developed in these animals. Naked DNA vaccination can enhance immunity to these gene products and thus intervene in disease regulation (Figs. 1, 5, and 6).

Administration of cytokine/hormone-encoding gene in a plasmid (i.m.) might be used as a way of eliciting the production of its gene product, which can be used for targeted delivery of gene products (23–25), or as a way to elicit an immune response against the gene product encoded by this construct (10, 11, 26, 27). The mechanistic basis leading to these opposing effects is not fully understood yet, and may be dependent, in part, by the nature of the selected plasmid. The existence or absence of repeated immunostimulatory sequence (CpG) may provide a partial explanation for the ability of certain plasmids to serve as adjuvants (for a very recent review, see Gurunathan et al.) (28). In our experience, administration of inflammatory chemokines and TNF-α-encoding constructs ligated into the pcDNA3 plasmid always resulted in the generation of anti-self immunity that was turned on during an autoimmune process (10, 11).

The current study shows that either neutralizing Abs (self-specific) or a targeted DNA vaccine encoding TNF-α can markedly reduce, yet not totally abolish AA (Figs. 1, 4, and 5). On one hand, these results are remarkable since they imply that targeting the function of a single proinflammatory cytokine exerts a profound effect on a disease regulated by various cytokines and chemokines (29–37). On the other hand, we (current work) and others could not use either targeted DNA vaccines, Abs to TNF-α, or soluble TNF-α receptor to totally eradicate the disease (38–40). The clinical implications of these findings are highly important since successful clinical trials are now being conducted using either TNF-α-specific Abs or TNF-α soluble receptor. One way by which these results could be interpenetrated relies on the hypothesis that at the clinical readout of disease resides from a balance between the activity various proinflammatory cytokines/chemokines such as TNF-α, IL-1, RANTES, MCP-1, and regulatory cytokines such as IL-10 and IL-4. Thus, blockade of the function of one proinflammatory mediator would significantly alter this balance, yet this effect is limited since other cytokines can overcome, in part, this absence. Alternatively, it could also be that neutralizing TNF-α by each of the above means does not totally abolish TNF-α functions.

Neutralizing the activity of TNF-α by Abs or even by soluble receptor therapy as a way to treat arthritis has been explored by...
several investigators, and is now in clinical trials. However, a major disadvantage in treating chronic diseases with xenogenic neutralizing Abs lies in their immunogenicity. This has motivated investigators to develop chimeric humanized Abs, and mAbs engineered with human Ig heavy and light chain yeast artificial chromosome (41). However, following repeated immunization, these engineered Abs can trigger allotypic responses. The therapeutic strategy suggested in this study is of advantage over the above methods since it resulted in the generation of immunity to autologous Ags that accelerates during the course of an autoimmune condition in accordance with disease progression. Even though this makes this type of therapy a very promising means of treatment for rheumatoid arthritis, and possibly other T cell-mediated autoimmune diseases (10, 11), one should also be aware of its limitations. Patients developing a chronic form of disease will have to spend the rest of their lives with Abs to their own TNF-α.

The long-term experience gained in clinical trials that are now being conducted, in which patients are continually subjected to soluble TNFR or Abs to TNF-α, is highly important for this perspective. Only if it would appear that long-term neutralization of TNF-α does not lead to harmful side effects should TNF-α-encoding DNA vaccine gene therapy be considered. Another obstacle is the differences in effectiveness of vaccines between animal models and humans. Yet, many efforts are being spent on improving the effectiveness of DNA vaccines in humans.

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References


