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DNA Immunotherapeutics: New Potential Treatment Modalities for Allergic Disease

Abstract

Genetic immunization is a relatively new approach to vaccination, one that has generated considerable interest for its potential to prevent or treat a number of types of disease processes. Although initial work focused on potential applications in infectious disease, it was soon recognized that the antigen-specific Th1 response typically generated by genetic immunization protocols could also be useful for the treatment of allergic disease. In this review we present a summary of genetic vaccination and the related topic of immunostimulatory DNA sequences – which we collectively designate DNA immunotherapeutics – and discuss their potential for the prevention and therapy of allergic disease.

Overview of Gene Vaccines

Genetic vaccination, the elicitation of an immune response against an antigen that is encoded by an injected plasmid (p) DNA (and synonymous with DNA vaccination), has only recently been described. In 1990 it was shown that naked pDNA injected into murine muscle can be taken up by local cells and that the encoded gene product can be expressed for at least several months [1]. Shortly thereafter, similar results were obtained by bombarding various tissues with pDNA-coated microprojectiles [2]. Reports that this phenomenon could induce an immune response against the pDNA-encoded antigen [3] and that these responses could protect the host against infectious pathogens [4–8] were not long to follow. Notably, the immune response generated against the experimental antigens was characterized by not only a humoral component, as is seen with traditional vaccines, but by a cellular one also, manifested by antigen-specific cytotoxic T lymphocytes [7]. Since then, the response has been shown to be long lasting, having persisted for greater than 12 months in monkeys and rodents [8–10]. It has further been shown to be elicitable to a number of antigens, such as hemagglutinin [4–8],

nucleoprotein and matrix protein [11] from influenza virus, gp-120 and -160 from HIV-1 [8], gIV from bovine herpesvirus [12], surface glycoprotein from rabies virus [13], hepatitis B virus surface Ag [14], malaria circumsporozoite protein [15], hsp 65 from mycobacteria tuberculosis [16], and hepatitis C virus core antigen [17]. Experiments have been successfully conducted in a broad range of different animal species including mice [7], rats [18], rabbits [18], chickens [4], ferrets [19], cattle [12] and nonhuman primates [20], bearing out the generalizability of the phenomenon. The use of the technique has not been limited to the field of infectious disease, as it has also been utilized to successfully suppress autoimmune [21] and allergic responses [22–24], as well as to generate antitumor [25], anti-idiotypic [26], and alloimmune responses [27].

While genetic immunization holds significant promise with respect to these multiple potential applications, it is a technique that is inherently simple. For the end-user, genetic vaccination merely requires injecting, either intramuscularly (i.m.) or intradermally (i.d.), pDNA that is suspended in saline and that contains an antigen-encoding gene sandwiched between a strong viral promoter and an efficient polyadenylation/transcriptional termination sequence [28].

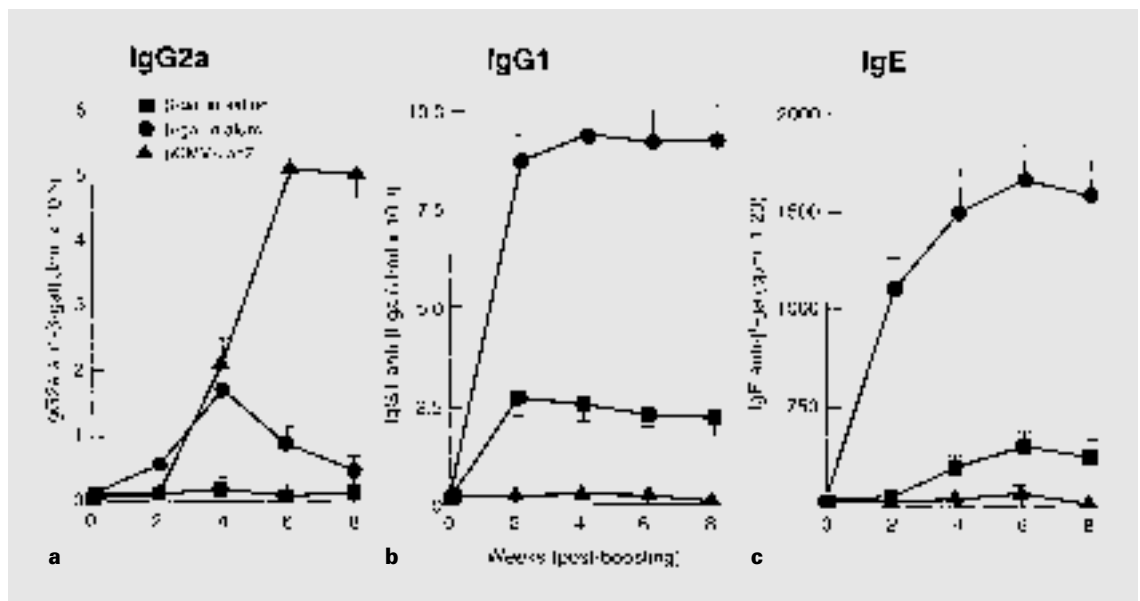


Fig. 1. The primary IgG2a, IgG1 and IgE (a–c) response of BALB/c mice injected with 100 µg of pCMV-LacZ, 10 µg of β-gal in saline or 10 µg of β-gal in alum. Results are mean + SE of four mice per group. Reproduced with permission from Raz et al. [22].

This simple technique can lead to long-lasting intracellular expression of the transgene product, reported as late as 19 months after injection in one study [29]. In our lab, histologic examination of the skin inoculated i.d. with a pDNA encoding either influenza nucleoprotein or *Escherichia coli* β-galactosidase (β-gal) in a human cytomegalovirus (CMV) expression vector have shown a variety of cells, both somatic and bone marrow-derived, to have been transfected, including keratinocytes, fibroblasts, and cells with the morphology of macrophages and dendritic cells. Intramuscular injection, on the other hand, predominantly transfects myocytes [1]. Recent work has demonstrated that bone-marrow-derived cells, such as professional antigen presenting cells (APCs), and not somatic cells, are required for the generation of the subsequent immune response to the pDNA-encoded antigen [30]. Given this and the fact that the dermis is a tissue with a rich supply of APCs, such as macrophages, dendritic cells and Langerhans cells [31], we usually employ the i.d. route of administration, but certainly the i.m. route has also been shown to be effective at eliciting an immune response in numerous studies [28]. Bombardment of tissues with DNA-coated microprojectiles using a ‘gene gun’ has also been employed extensively, but it has been reported that this mode of inoculation typically produces a Th0/Th2 response, in contrast to the Th1 response characteristically seen when using the i.d. or i.m.

routes [32]. Indeed, this ability to induce a Th1 response is the most intriguing facet of genetic immunization from the perspective of allergic disease. As has been previously reported [33], gene vaccines administered i.d. or i.m. produce a Th1 response against the encoded antigen. We shall here summarize a series of experiments which clearly demonstrate this phenomenon.

Gene Vaccines Induce an Antigen-Specific Th1 Primary Response

Immunoglobulin isotypes were compared in mice that had been vaccinated in one of three different ways [22]: mice were immunized i.d. with β-gal protein in saline (β-gal/saline), β-gal protein in alum (β-gal/alum) or with pCMV-LacZ, a plasmid construct containing the gene encoding β-gal under the control of the human CMV promoter. The humoral immune response was then characterized by serially assessing the antigen-specific immunoglobulin (Ig) isotype among each of the experimental groups (fig. 1). The protein-immunized mice, particularly those receiving alum, a known potent inducer of Th2 responses, produced high levels of β-gal-specific IgG1 (an isotype associated with Th2 responses) and very low titers of IgG2a (a Th1-associated isotype). Conversely, the pDNA-immunized mice

had the reverse pattern, showing high IgG2a and minimal IgG1 production. Using an alternative antigen (influenza nucleoprotein) and corresponding pDNA (pCMV-NP), identical IgG isotype patterns were observed, demonstrating that this phenomenon was not specific for the β -gal system. When the titers of IgE (another Th2-associated antibody) were assayed in the various experimental groups, the β -gal/alum group produced high, sustained titers of antigen specific IgE over at least 8 weeks. β -gal/saline produced only weak levels, while pCMV-LacZ did not induce detectable titers of anti- β -gal IgE. Assessment of total IgE levels showed that pDNA-vaccinated mice had extremely low levels compared to the levels of the β -gal/alum-injected or those of the β -gal/saline group.

Cytokine production by splenocytes taken from mice subjected to the above experimental conditions and then restimulated in vitro with β -gal was also measured (table 1). The splenocytes from the β -gal/saline group released moderate levels of interleukin (IL)-4 and IL-5, and the β -gal/alum group produced very high levels of these Th2 cytokines, while both produced undetectable levels of the Th1 cytokine interferon- γ (IFN- γ). The reverse was found for the pCMV-LacZ group, with undetectable levels of IL-4 and IL-5 and very high levels of IFN- γ production. These cytokine data confirmed the findings of the antibody isotype data: protein vaccination, particularly with alum as an adjuvant, produces a primary Th2 response, while vaccinating with pDNA results in a Th1 response.

Gene Vaccines Inhibit the Induction of a Secondary Th2 Response

Given that DNA vaccination elicits such a polarized primary response, could the primary Th1 response persist despite an antigenic exposure that would normally induce a Th2 response? Using mice that were primed with pCMV-LacZ or β -gal/saline, an attempt was made to boost with β -gal/alum and measure the subsequent humoral immune response (fig. 2). The IgG2a response to these regimens was very strong in the pCMV-LacZ-primed group and negligible in the β -gal/saline-primed mice. As expected, the β -gal/alum boost induced a strong IgG1 response in the mice primed with β -gal/saline that was roughly twice that of the mice primed with pCMV-LacZ. The IgE response by the protein/alum boost, however, was negligible in mice primed with the gene vaccine, and strong in those primed with protein. Furthermore, these results were antigen-specific, as mice primed with pCMV-LacZ and then boosted with an unrelated protein antigen (OVA) in alum produced a robust

IgE response specific for OVA but negligible levels of anti- β -gal IgE. These findings suggested that once a Th1 response is established in response to a primary antigenic exposure in the form of a gene vaccine, the Th1 response will persist despite a secondary exposure to a strong Th2-inducing stimulus (β -gal/alum).

Genetic Vaccination Induces a Switch From a Primary Th2 to a Secondary Th1 Response

Nevertheless, the more clinically relevant question is: can an ongoing Th2 response be converted to Th1 using pDNA immunization? To answer this, we next evaluated the immune response generated by boosting with pCMV-LacZ mice that had been primed with β -gal/alum and therefore had already generated a primary Th2 response. These results are summarized in figure 3. Mice primed with β -gal/alum and then boosted with pCMV-LacZ, as compared to those boosted with β -gal/saline showed a marked inhibition of IgE and IgG1 production, while producing a vigorous IgG2a response. As with the previous group of experiments, the response was antigen-specific. When we measured cytokine production (table 1) by β -gal-stimulated CD4⁺ cells taken from these mice that were primed with β -gal/saline and boosted with the matching gene vaccine, IFN- γ levels were high, similar to those of the pCMV-LacZ-primed mice. IL-5 levels were one-third of control levels (vs. β -gal/alum priming only), while IL-4 levels were unchanged. Given that IL-4 levels were stable and IFN- γ levels were markedly elevated, the decreased IgE titers can likely be explained by the increased production of the latter cytokine. Taken together, these data showed that pDNA immunization can shift an ongoing Th2 response to one that is phenotypically Th1.

Genetic Vaccination Inhibits Pulmonary Eosinophilia

Eosinophilia is an important component of the atopic response and pulmonary eosinophilia figures prominently in the pathophysiology of allergic asthma [34]. A further set of experiments was therefore carried out to evaluate the ability of genetic vaccination to inhibit pulmonary eosinophilic inflammation [35]. The hen egg OVA gene was subcloned to a CMV promoter-driven vector, generating pCMV-OVA. To effect immunologic priming, this plasmid or a control that did not encode OVA was injected i.d. into BALB/c mice. Animals were then Th2-sensitized to OVA by injecting the

Fig. 2. The IgG2a, IgG1, and IgE response to secondary immunization with 1 µg of β-gal in alum of mice primed with either 100 µg of pCMV-LacZ or 10 µg of β-gal in saline (**a-c**). To demonstrate antigen specificity, an additional group of pCMV-LacZ-primed mice were injected with 2 µg of OVA in alum (**d**). Results are mean + SE of 4 mice per group. Reproduced with permission from Raz et al. [22].

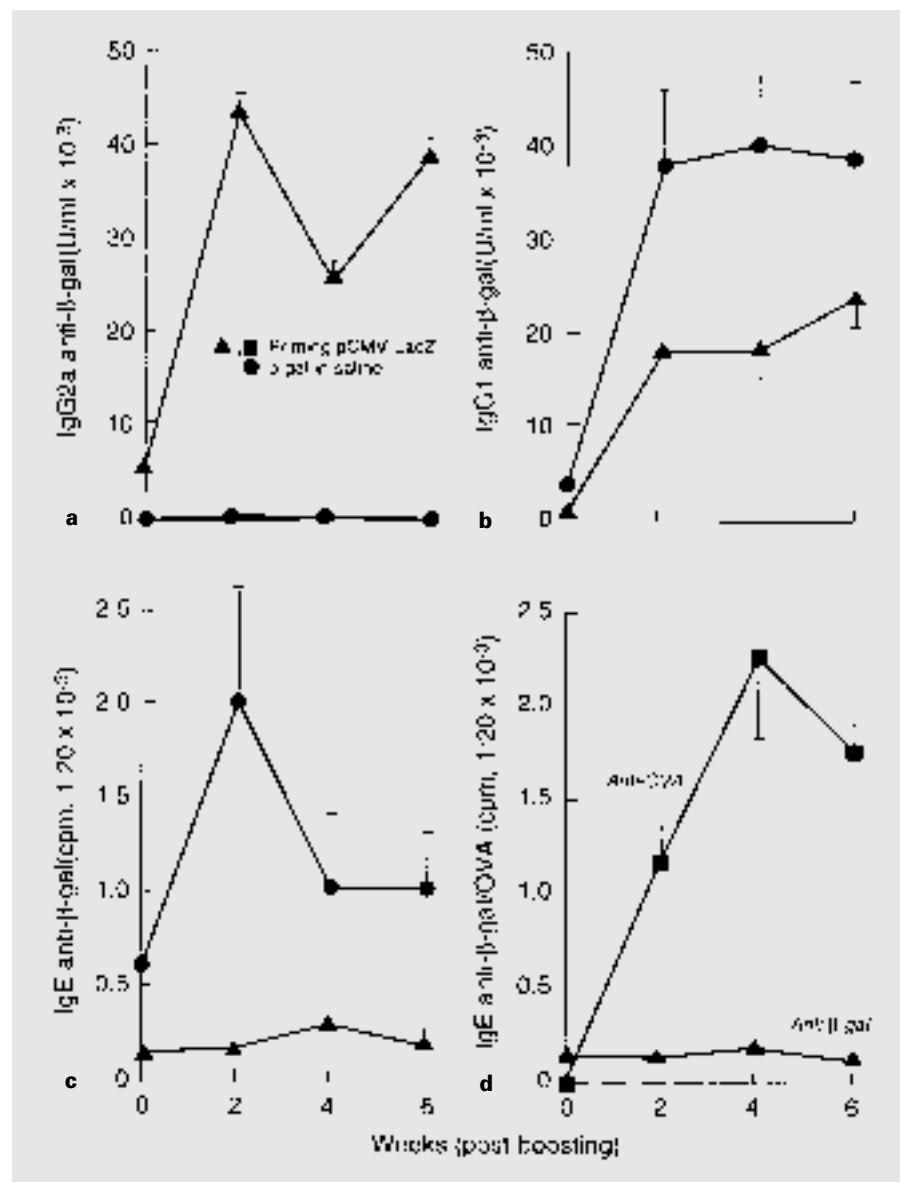


Table 1. Cytokine production by β-gal-stimulated CD4⁺ splenic T cells from pDNA and/or protein immunized mice

Experiment	Priming	Boosting	IFN-γ pg/ml	IL-4 pg/ml	IL-5 units/ml
A	None	None	<10	<2	<2
B	pCMV-LacZ	None	741±170	<2	<2
C	β-gal/saline	None	<10	12±2	15±4
D	β-gal/alum	None	<10	351±24	642±51
E	pCMV-LacZ	β-gal/alum	1050±314	4±2	46±10
F	β-gal/saline	β-gal/alum	54±48	126±43	165±54
G	β-gal/alum	pCMV-LacZ	730±193	307±128	183±70

pCMV-LacZ mice were injected with 100 µg of pDNA encoding β-galactosidase (β-gal). β-gal/alum mice received 10 µg β-gal protein in alum. Cytokines were measured by enzyme-linked immunosorbent assay using Biosource kits or reagents. Values are mean ± SE of splenic CD4⁺ cells from 4 mice per experiment. Reproduced with permission from Raz et al. [22].

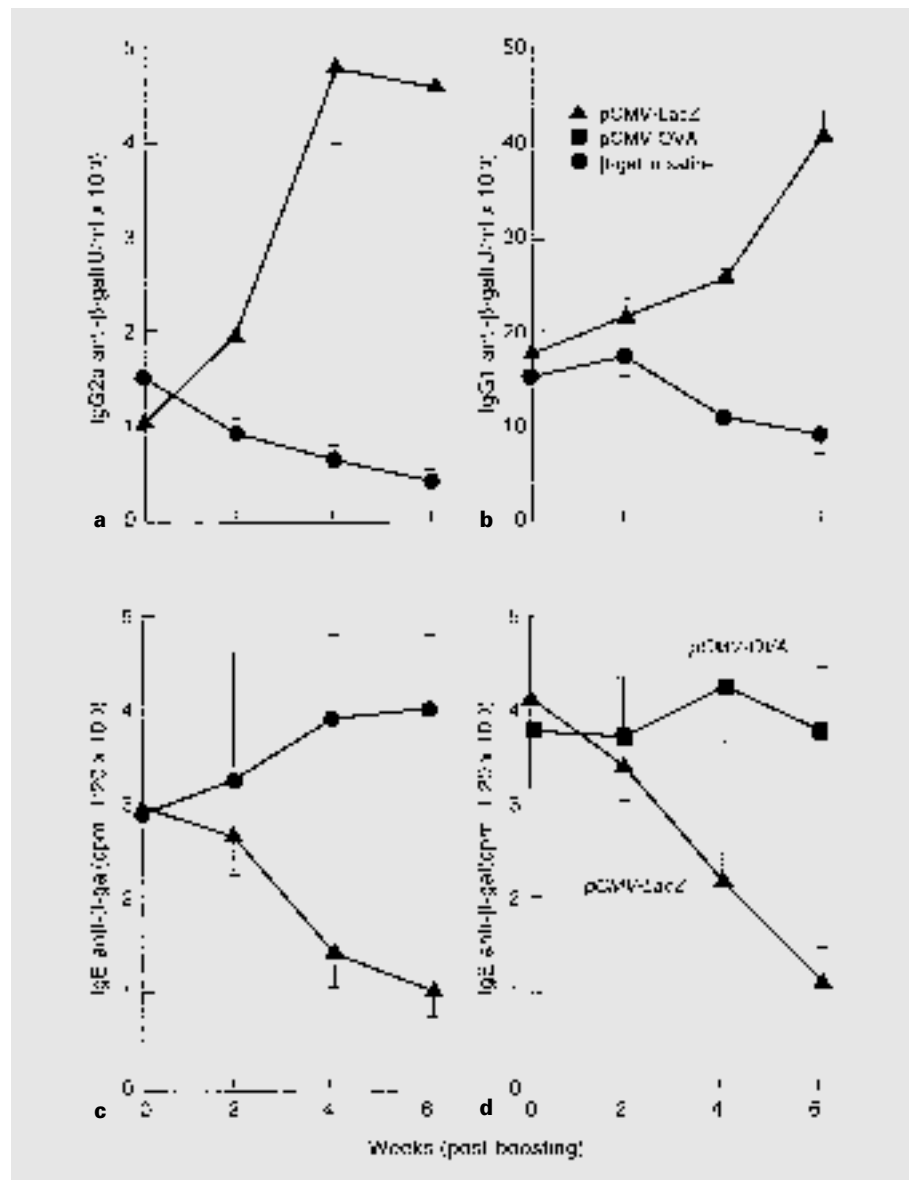


Fig. 3. The IgG2a, IgG1, and IgE response to a secondary immunization of either 100 µg of pCMV-LacZ or 1 µg of β-gal in saline, injected i.d. into mice primed with 1 µg β-gal in alum (**a-c**). To demonstrate antigen specificity, control mice were injected with 100 µg of pCMV-LacZ (**d**). Results are mean + SE of 4 mice per group. Reproduced with permission from Raz et al. [22].

protein in alum subcutaneously (s.c.) and subsequently were challenged with aerosolized OVA inhalation treatments. To evaluate the effect of OVA gene vaccination on the induction of the late-phase-reaction component of the pulmonary allergic response, the animals were sacrificed 24 h after the last OVA inhalation [36], and eosinophils were quantitated in the peripheral blood, bone marrow, bronchoalveolar lavage (BAL) fluid and in lung tissue. As demonstrated in table 2, the injection of pCMV-OVA inhibited eosinophilic infiltrates in the BAL fluid, lung tissue, and bone marrow, while the injection of normal saline or the control plasmid (pUC19) did not. Thus, the injection of pDNA encoding an allergen can not only cause a shift to-

ward the secretion by CD4⁺ cells of Th1 cytokines, and decrease IgE and IgG1 production while upregulating IgG2 production, but can also decrease the proliferation and/or recruitment of eosinophils to tissues where allergic eosinophilia is commonly found.

Similar results have been obtained by Hsu et al. [37] with respect to pulmonary immediate hypersensitivity, using a different allergen (Der p 5) and a rat model instead of the murine model we have used. By injecting pDNA encoding Der p 5, this group was able to inhibit histamine release in bronchoalveolar lavage fluid and airway hyperresponsiveness in rats that were challenged with aerosolized allergen. This treatment regimen also inhibited specific IgE syn-

Table 2. pDNA immunization inhibits pulmonary eosinophilic inflammation

Priming	Sensitization	Challenge	Blood	BM	BALF	Lung
(1) Saline	+	+	5.0±2.4	10.2±1.8	39.5±5.0	40.4±15.8
(2) OVA-pDNA	+	+	4.0±0.8	3.1±0.7	6.2±1.8	12.0±1.4
(3) pUC19	+	+	8.1±0.7	8.4±0.5	32.2±7.2	38.5±1.7
(4) Naive	–	–	7.4±2.0	3.7±0.5	0±0	0±0

Differential counts (%) of eosinophils in various tissues after OVA inhalation. OVA gene vaccines (50 µg OVA-pDNA administered i.d.) given prior to exposure to repeated OVA/alum administrations (groups 2 and 3), inhibited the subsequent eosinophil infiltrate in the bone marrow (BM), in the bronchoalveolar lavage fluid (BALF), and in the lung tissue (Lung) after inhalation challenge with OVA (8 mice per group). pUC19 is a non-OVA-encoding control plasmid. Eosinophil cell counts were performed by a 'blinded' investigator on slides coded with numbers. Five hundred white blood cells were counted in the BALF of each mouse and ten random light microscope fields of lung sections were analyzed for determination of eosinophil counts in lung tissue. Results are mean ± SE.

thesis. The same group has also demonstrated decreased IgE levels in mice that were injected intraperitoneally (i.p.) with Der p 5 after the prophylactic i.m. injection of their Der-p-5-coding plasmid [23]. Another report has shown inhibition of IgE in mice via vaccination with a plasmid coding for Hev b 5, a latex allergen [38]. Using different model allergens and different animal models, gene vaccination has demonstrated significant promise for the prevention or treatment of the allergic response [22, 37, 39].

Immunostimulatory DNA Sequences

Since the early reports of the ability of genetic immunization to induce immune responses, much speculation has been posited regarding the mechanisms of the phenomenon. In particular, how does this vaccination strategy produce a Th1 response, while inoculation with protein antigen produces a Th2 response? Initial arguments held that in gene vaccination since the gene product was intracellularly produced, it would, much like viral proteins, be processed as an endogenously produced cellular protein and be presented to the immune system via the MHC class I presentation pathway. This argument certainly makes sense teleologically – genetic vaccination essentially mimicked a viral infection, thereby inducing an analogous immune response to such an infectious process. This hypothesis, however, is significantly lacking in mechanistic detail. Immunostimulatory sequences (ISS) of DNA, on the other hand, have recently been shown to play an instrumental role in gene vaccination, and now figure prominently in an alternative hypothesis.

ISS are short DNA sequences (on the order of 6 bp) containing an unmethylated CpG motif (i.e. covalently linked cytosine and guanine nucleotides, not a C:G base pair). Despite the fact that the biochemical and genetic properties of DNA have been, and continue to be, thoroughly investigated, only recently has it been appreciated that DNA carries more information than simply the blueprints for the regulation and construction of proteins. There have now been several reports that bacterially derived ISS stimulate the immune system to mount robust, Th1-type immune responses, and that they furthermore play a fundamental role in the generation of the immune response that is seen in genetic immunization [40–42].

A variety of polynucleotides have been shown to be immunostimulatory. For instance, dsRNA or its analogue poly-inosinic, poly-cytidylic ribonucleic acid (poly I:C), can induce IFN-α and -β, thereby inducing an antiviral response [43, 44]. It is thought that this response (now known to be mediated by the IFN-induced, dsRNA-activated protein kinase (PKR) [45, 46] evolved to recognize dsRNA due to its association with viral infection. Polyguanosinic, poly-cytidylic deoxyribonucleic acid (poly G:C) can induce lymphocyte proliferation [47, 48] as can bacterial DNA, while vertebrate DNA possesses no such activity [48].

Tokunaga and colleagues [49, 50] were the first to show that naturally occurring DNA could be immunostimulatory when they found that the nucleic acid fraction of *Mycobacterium bovis* BCG has potent antitumor activity, that the DNA is solely responsible for the effect, and that the DNA is not directly cytotoxic, signifying that the effect is host mediated. This group also showed that bacterial DNA stimulated both macrophages and NK cells, which could be in-

hibited by anti-IFN- γ and anti-IFN- α/β antibodies, respectively. By synthesizing oligodeoxynucleotides (ODNs) from different regions of the mycobacterial genome, 6 (out of 13) single-stranded 45-mer ODNs were identified that activate macrophages and NK cells, cause antitumor activity, and induce the production of IFN- α/β and IFN- γ . An analysis of shorter, overlapping ODNs and the insertion of active sequences into otherwise inactive ones, enabled them to attribute the immunostimulatory activity to palindromic hexamers, each containing a CpG dinucleotide [51–55]. Using a murine B cell proliferation model, Krieg et al. [56] found that these sequences were optimally mitogenic when following the formula 5'-purine-purine-CpG-pyrimidine-pyrimidine-3'. The crucial stimulatory component of the DNA sequences was the CpG dinucleotide – if it is removed [56] or methylated [35, 56–59], the immunostimulation is abolished. Subsequent studies showed that CpG-enriched ODNs also induce the secretion of IL-6 and IL-12 from B cells [60] and IFN- γ from NK cells [57]. Interestingly, it has since been shown that, in contrast to previously published studies [51–55], the CpG motif does not absolutely have to be placed in the context of a palindromic hexamer. Departing even farther from the consensus motif, a recent report suggests that certain sequences lacking CpGs can retain some immunostimulatory properties, albeit at suboptimal levels [61]. Nevertheless, CpG-containing sequences have now been shown by several different groups to have robust immunostimulatory properties.

That ISS play an important role in the induction of the immune response seen in gene immunized animals is now coming more clearly into focus. It has been previously reported that the addition or deletion of various ISS to or from different pDNAs can dictate the nature of the immune response of the pDNA-encoded gene product [41]. This serendipitous finding stemmed from the observation that two plasmids, both encoding β -gal and similar except for the antibiotic selection marker – one containing an ampicillin resistance gene (amp^r), the other containing a gene conferring resistance to kanamycin (kan^r) – generated markedly different immune responses. When injected into mice, the pDNA containing amp^r , known as pACB-Z, produced a typically robust Th1 response. On the other hand, injection of pKCB-Z, the kan^r containing plasmid, resulted in only a meager response. Analysis of the sequences of the two resistance genes revealed that the amp^r gene contained several hexamers that followed the 5'-pur-pur-CpG-pyr-pyr-3' model. Kan^r , meanwhile, contained none of these CpG-containing ISS. When ISS were subsequently subcloned into pKCB-Z, the response was restored to a magnitude that was similar to that of pACB-Z. Similarly, the re-

sponse could also be restored by coinjecting a second non-coding plasmid that contains ISS along with a coding plasmid that does not have ISS [61]. These results illustrated that the plasmids used in DNA immunization can be conceptualized as having two distinct and important subunits – a component that codes for antigen and a second portion that provides the needed immunostimulatory signals.

ISS Elicit a Th1-Inducing Cytokine Milieu

We now have some insight into the nature of those immunostimulatory signals. Transfecting fresh human macrophages with ISS-containing DNA (either ISS-ODN, or pDNA containing ISS), but not with ISS-deficient DNA (mutated (M)-ODN in which the CG dinucleotide is replaced with GG, or pDNA lacking ISS), results in increased production of mRNA transcripts for IFN- α , IFN- β , IL-12 and IL-18 [42]. All of these cytokines are known to induce synthesis of IFN- γ [41, 62–66], and play a role in the differentiation of naive Th cells to Th1 phenotype. Furthermore, incubating human macrophages with IFN- α produced increased transcript levels of IL-12 and IL-18, which suggests that IFN- α serves as an amplifier in ISS-induced stimulation, and potentially in Th1 induction more generally. Additionally, transfection of human peripheral blood mononuclear cells with ISS-ODN, but not with M-ODN, results in the production of IFN- α , IFN- β , and IL-12 by macrophages [42] and of IFN- γ by natural killer cells [42, 67]. ISS, therefore, induce cells of the innate immune system – independent of the presence of antigen – to produce a cytokine milieu that promotes a Th1-type response when antigen is present.

The ISS-stimulated immune response can therefore be thought of as consisting of two distinct phases – an antigen-independent phase consisting of the response of the innate immune system to the presence of ISS and an antigen-dependent phase involving the adaptive immune response (fig. 4). In the former, the presence of ISS stimulates the innate immune system to produce IFN- γ and inducers of IFN- γ (IFN- α , IFN- β , IL-12, IL-18), and sets up a cytokine milieu capable of biasing naive Th cells toward Th1. The second phase occurs when Th cells and B cells, already under the influence of the Th1-inducing cytokines from phase 1, encounter antigen and begin producing IFN- γ and Th1-associated antibodies, this time in an antigen-dependent manner. This second phase of the response results in IFN- γ levels that are greater than those of the first phase (ng/ml compared to pg/ml, measured *in vitro*).

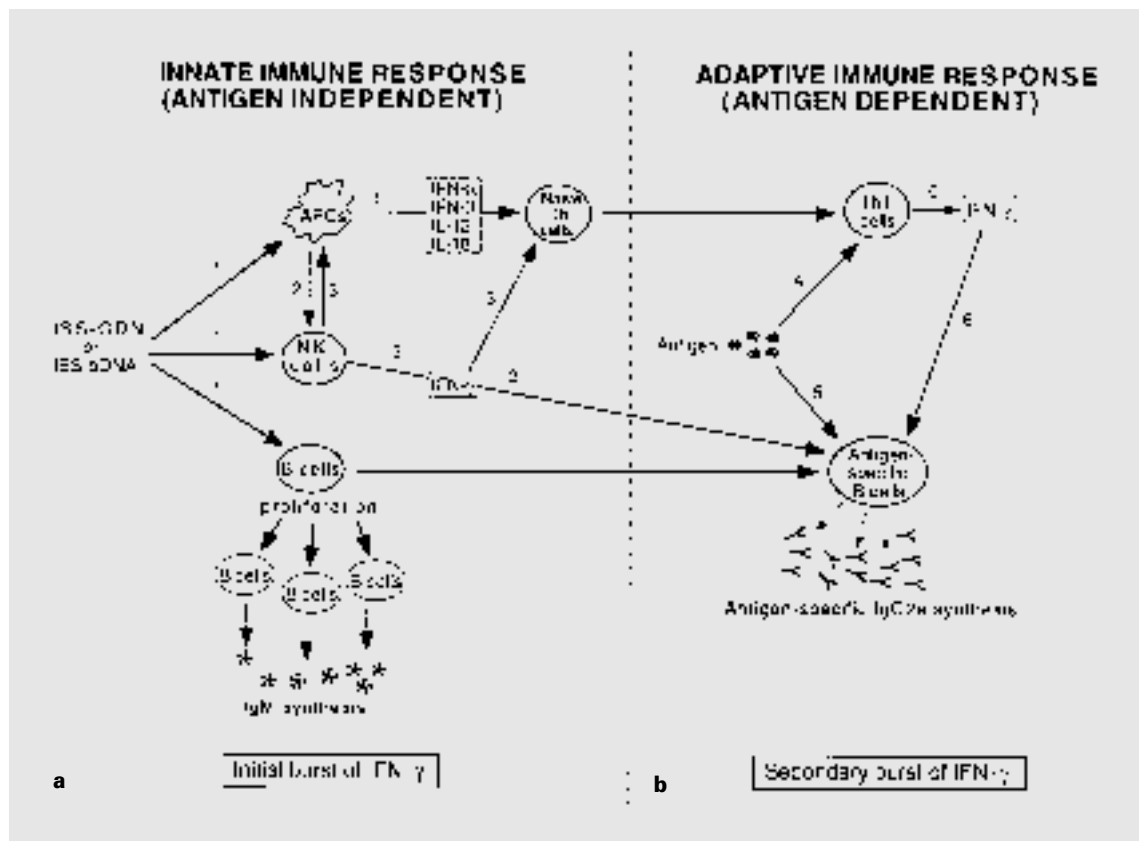


Fig. 4. A postulated mechanism of the induction of a Th1 response by gene vaccination. **a** In an innate immune response (involving an initial burst of IFN- γ) ISS-ODN or ISS-pDNAs trigger the release of IFN- γ by NK cells and inducers of IFN- γ (such as IFN- α , IL-12, and IL-18) by APCs (e.g. macrophages), and act as mitogens for B cells (step 1). The release of IL-12 by APCs results in further IFN- γ production by NK cells (step 2). Production of IFN- γ by NK cells acts on both naive T cells and on B cells (step 3). The initial burst of IFN- γ , as measured in vitro, is in the pg/ml range. **b** In the second phase (the adaptive immune response) in the presence of antigen (involving a secondary burst of IFN- γ), naive Th cells are primed by the cytokines secreted by the innate immune system to differentiate towards Th1 cells (step 4). Encounter with antigen under the influence of secreted innate cytokines induces B cells to class switch and produce IgG2a antibodies (step 5). Th1 cells produce additional IFN- γ in an antigen-dependent fashion (Th1 response) (step 6). The secondary burst of IFN- γ , as measured in vitro is in the ng/ml range. Reproduced with permission from Tighe et al. [33].

This model is borne out by experiments from our lab [42] and by Chu et al. [68] showing that ISS can induce a robust Th1 response to a co-administered protein antigen. Protein that is co-injected with ISS-containing DNA (ISS-ODN or pDNA containing ISS), in contrast to co-injection with ISS-deficient DNA, results in a murine immune response characterized by production of antigen-specific cytotoxic lymphocyte activity [J.S. Goodman & E. Raz, unpublished data], IgG2a antibodies, and IFN- γ [42, 68] – the hallmarks of a Th1 response. Conversely, Chu et al. [68] report that IL-5 production, associated with Th2 responses, was diminished when ISS and protein were co-administered. Interestingly, protein/ISS-DNA coinoculation also re-

sulted in the production of IgG1 [42, 68] and IL-4 [42] levels that were comparable to those of protein/ISS-deficient DNA coinoculation. Yet, when mice were primed with either regimen and then challenged with protein in alum, antigen-specific IgE levels were dramatically inhibited in those mice receiving ISS-DNA compared to those receiving ISS-deficient DNA.

These findings are not inconsistent with the above-postulated model, because even though ISS/protein coinjection gives a less decisive Th1 response than genetic vaccination, correlates of the polarized Th2 response of allergy (such as IgE) are still inhibited. One potential reason for the differences between these modalities is that the much greater

dose of antigen employed in these experiments (in the microgram range) likely results in a broader anatomic distribution of antigen, some proportion of which is likely to be beyond the local influence of the coinjected ISS. This is in contrast to the situation seen in genetic vaccination in which the amount of gene product is much smaller (pg/ml to ng/ml) [69] and produced in close proximity to the immunostimulatory cytokine milieu induced by the transfected ISS. Another notable finding of this series of experiments was the observation that overall antigen-specific IgG titers, which could function as allergen-blocking antibodies, are much greater than those obtained by the matching gene vaccine. This observation, coupled with the finding of inhibition of IgE upon protein/alum challenge suggests considerable potential for the application of protein/ISS co-injection to the treatment of allergy.

Protein/ISS Coinjection Inhibits Pulmonary Eosinophilia

Indeed, in a recent series of as yet unpublished experiments carried out in our lab, we have further examined this coinjection strategy by assaying its ability to inhibit a pathophysiologic hallmark of allergic asthma – pulmonary eosinophilia. We have found that oligonucleotides (ODNs) containing these ISS can profoundly inhibit allergic eosinophilic inflammation in a mouse model of the late phase reaction of allergy. Allergen-sensitized mice were administered ISS-ODN or control treatments before challenge with aerosolized allergen, and examined for the presence of eosinophils in the lungs, blood, and bone marrow. Remarkably, pretreatment via the intraperitoneal, intranasal, or intratracheal routes with ISS-ODN before challenge nearly completely inhibited eosinophilic infiltration in contrast to the marked eosinophilia seen in the controls. For example, in the intraperitoneal study, sensitized but untreated mice and those that received an ODN in which the ISS was mutated to remove its stimulatory activity (via exchange of the CG for GG) suffered a severe late-phase response, with eosinophils comprising 43% of the nucleated cells in the bronchoalveolar lavage fluid. However, those animals receiving ISS-ODN were protected, with only 3% eosinophils (vs. 0% in nonsensitized, nonchallenged mice) – a 93% reduction in the allergic response (Broide et al., submitted for publication). This effect is even stronger than daily dexamethasone pretreatment.

When restimulated in vitro with allergen, CD4⁺ T cells from these ISS-ODN-treated mice produce less IL-5 and more IFN- γ than controls, indicating an allergen-specific

shift from a Th2 dominated response to Th1 (submitted). These observations further support our hypothesis that the primary anti-allergic activity of gene vaccination is attributable to the ISS motifs present within the vector plasmid. In this study, the antigen was not produced intracellularly (as in pDNA vaccination), but was instead delivered exogenously as protein, arguing against the requirement for intracellular production for development of a Th1 response. Additionally, the only DNA employed in this study was a short oligonucleotide encoding the ISS, demonstrating that this alone is sufficient for induction of the specific Th1 response, even when given prior to antigen delivery. One exciting implication of this data is that one could potentially prime allergic patients with ISS and then allow them to go into their typical allergen-laden environment in order to reeducate their immune system to deal with the allergens in a Th1, rather than Th2, manner. This strategy could conceivably obviate the need for even identifying the allergens to which a given patient was sensitive.

DNA Immunotherapeutics: A Possible Alternative to Immunotherapy

DNA immunotherapeutics – comprising genetic vaccination and ISS/protein coimmunization – holds great promise as a potential treatment modality for a disease that is sorely in need of treatment alternatives. At present, treatment regimens are comprised of allergen immunotherapy (IT) and/or pharmacologic intervention, both of which have significant drawbacks. IT, a process in which gradually increased doses of purified allergen are subcutaneously administered to patients suffering from allergies in an effort to downregulate their allergen hypersensitivity (perhaps by switching the response from Th2 to Th1 dominated), has been utilized since the early part of the century [70]. Nevertheless, despite millions having undergone this treatment over the ensuing years [71], its role in the treatment of allergic disease remains controversial. At present, it is generally agreed upon that IT is indicated for the treatment of venom hypersensitivity and, in some cases, allergic rhinitis, but the utility and safety of IT in the treatment of asthma and especially food allergy have recently been questioned [72, 73]. The small risk of anaphylaxis with IT is a serious concern. One study, for instance, showed the rate of systemic reaction seen over the course of 500,000 IT injections to be 2.9% [74], while another survey reports 17 deaths due to IT in the United States between 1985 and 1989 [75]. A 1996 *New England Journal of Medicine* editorial [73] included this severe indictment of the modality as a treatment for allergic asthma:

'There can be few indications for the use of allergen immunotherapy in the routine management of asthma in adults and children, in view of its low efficacy, the risk of adverse effects, the relatively high cost of treatment, and the availability of safer and more effective drug therapies, particularly inhaled glucocorticoids.'

The pharmacologic alternatives suggested by the author, however, require chronic therapy and high patient compliance, do not address the underlying immune dysregulation, are still relatively expensive, do not completely suppress symptoms, and carry side effects of their own. Even one of the safest and most effective therapies, inhaled glucocorticoids, can still cause cataracts, growth retardation, and os-

teoporosis. Additional therapeutic modalities for allergy are needed. Recent evidence suggests the DNA-based immunization strategies may represent a viable alternative.

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