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Immunology

DNA immunization: Induction of higher avidity antibody and effect of route on T cell cytotoxicity

(genetic vaccine/affinity/intradermal/intramuscular)

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ABSTRACT **Immunizations of mice with plasmid DNAs encoding ovalbumin (OVA), human Ig, and hen egg lysozyme were compared with doses of soluble protein (without adjuvant) that induced similar IgG responses. The route of immunization influenced the magnitude of the antibody (Ab) response in that intradermal (i.d.) injection elicited higher IgG Ab levels than i.m. injection in both DNA- and protein-immunized mice. Although total IgG levels were similar to soluble protein controls, the avidity of the anti-OVA Abs generated by DNA immunization were 100- and 1,000-fold higher via the i.m. or i.d. route, respectively. However, despite the generation of high-avidity Ab in DNA-immunized mice, germinal centers could not be detected in either DNA- or protein-immunized mice. Examination of the IgG subclass response showed that IgG2a was induced by i.m. DNA immunization, coinciding with elevated interferon γ production, whereas a dominant and elevated IgG1 response, coinciding with detectable interleukin 4 production, was generated after i.d. immunization with DNA or soluble OVA and hen egg lysozyme but not human Ig protein. As expected, cytotoxic T cell (CTL) responses could be detected only after DNA immunization. I.d. immunization produced the strongest CTL responses early (2 weeks) but was similar to i.m. later. Therefore, DNA immunization can differ from protein immunization by its ability to induce rapid CTL responses and higher avidity Ab, both of which are advantageous for vaccination.**

Injection of mammalian expression plasmid DNAs directly into muscle (1) and skin (2) or facilitated with biolistic systems (3) results in the uptake of DNA and expression of the encoded proteins. Expression levels of the encoded protein are often below detection and have been estimated by reporter enzymes such as luciferase (lux) to be in the nanogram range (1). However, even this relatively low dose of protein is sufficient to produce long-lasting immune responses (4) to encoded antigens that are capable of protecting animals from a wide range of pathogens (for review see ref. 5).

The mechanisms underlying the induction of immune responses after DNA immunization are unclear. I.m. injection results in low-level transfection of myocytes (1) whereas intradermal (i.d.) injection may directly transfect antigen-presenting cells (APCs) (2, 6). Because myocytes express major histocompatibility complex class I at low levels and do not constitutively express class II or costimulatory molecules such as B7 (7), they appear unlikely candidates for the induction of antibody (Ab) or cytotoxic T cell (CTL) responses after i.m. DNA injection. The immunological consequences resulting from priming with different cell types and perhaps location of immune induction have not been fully resolved for DNA immunization, because the majority of the research to date has concentrated on vaccine efficacy. We sought to examine the effect of immunization with DNA as compared with soluble protein and to ascertain the contribution of route and dose on differences observed. In this study we immunized mice with DNA encoding the secreted antigens ovalbumin (OVA), human Ig (hIg), and hen egg lysozyme (HEL) or soluble protein and compared the Ab avidity and levels, cytokine production, and CTL responses.

MATERIALS AND METHODS

Mice. Female mice aged 6 to 8 weeks were used in all experiments and maintained in specific pathogen-free conditions.

Plasmids and Immunizations. OVA and HEL were inserted into an expression plasmid (CIGH) containing a cytomegalovirus (CMV) promoter, intron, and the bovine growth hormone polyadenylation signal. hIg was expressed under the control of the CMV promoter in the CDM8 vector. The major histocompatibility complex IE alpha chain promoter was kindly provided by Jan Allison (Walter and Eliza Hall Institute, Melbourne, Australia) and inserted into pIC19R via *Sac*I/*Pst*I digestion, and the CMV promoter and intron in CIGH-lux (CIGH containing the lux cDNA) were replaced with the IE promoter as a 1.9-kb *Bgl*II–*Pst*I fragment (the resultant plasmid was designated IE-lux). Plasmids for injection were prepared from *Escherichia coil* by polyethylene glycol precipitation as described (8) except that volumes of solution I, II, and III were adjusted such that pellets were resuspended in 50 ml of solution I for each liter of broth media used. Endotoxin was removed from plasmid preparations by four Triton X-114 phase separations (9), and DNA was stored at −20°C in normal saline until injected. The resultant plasmid preparations contained less than 10 units of endotoxin per mg of plasmid DNA as determined by the limulus amoebocyte lysate assay (BioWhittaker). Mice received 100 μg of plasmid DNA or the indicated quantity of soluble protein in normal saline i.m. in both quadriceps or i.d. at the base of the tail on day 0 and 14 of each experiment unless otherwise stated.

Ab Assays. Microtiter plates (Dynatech) were coated with OVA protein (A-5503, Sigma; 10 μg/ml in PBS), hIg protein (Intragam, CSL, Parkville, Australia; 10 μg/ml in PBS) or HEL (Boehringer Mannheim; 10 μg/ml in PBS) by overnight incubation at 4°C and washed four times with PBS to remove unbound antigen. Plates were incubated with serially diluted sera in blocking buffer (5% skim milk powder in PBS) overnight at 4°C. After washing five times with PBS to remove

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Abbreviations: Ab, antibody; APC, antigen-presenting cell; CTL, cytotoxic T lymphocytes; HEL, hen egg lysozyme; hIg, human Ig; i.d. intradermal; IL, interleukin; INF, interferon; lux, luciferase; OVA, ovalbumin.

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unbound Ab, plates were incubated with peroxidase-conjugated anti-mouse IgG, IgG1, IgG2a, or IgG2b Abs (Southern Biotechnology Associates) diluted in blocking buffer. After washing five times with PBS, the amount of bound Ab was determined by addition of substrate solution [0.1 mg/ml 3,3,5,5-tetra methylbenzidine (Sigma, T2885) 0.03% H_2O_2 in 0.1 M Na acetate, 0.1 mM EDTA, pH 6.0]. The reaction was stopped with 1 M H_2SO_4 and the OD read at 450 nm. Titers were defined as the highest dilution to reach an OD of 0.2.

To calibrate the IgG subclass ELISAs, plates were coated with IgG1, IgG2a, or IgG2b from mouse myelomas (10 μg/ml in 0.5 times PBS) overnight at 4°C, washed three times with PBS, and then incubated with serially diluted anti-mouse IgG subclass horseradish peroxidase-conjugated Ab. The dilution of each anti-mouse subclass Ab that gave identical absorbances in the ELISA were used subsequently.

The avidity of the anti-OVA Ab was determined by antigen competition as described (10), and the avidity was reported as the log of the concentration (10 mg/ml OVA was the highest concentration used) added to the well that resulted in a 50% binding inhibition of each immune sera control.

Cytokine Detection. Mice were immunized with DNA or soluble protein on day 0 and 14 and then boosted on day 28 with 10 μg of soluble OVA protein in PBS. Three days post-boosting, 1×10^6 splenocytes were incubated in tissue culture media (RPMI with 10% fetal calf serum) containing OVA protein for 48 hr. Supernatants from cultures were assayed for the presence of interleukin (IL) 4 and interferon (INF)-γ by sandwich ELISAs (PharMingen), and the lower limits of detection were 15 and 250 pg/ml, respectively.

CTL Assays. Splenocytes from mice were stimulated with irradiated (200 Gy) E.G7-OVA cells (EG7) (11) at a ratio of 10:1 for 5 days in 30 ml of tissue culture media. After washing with media, cells were counted and serially diluted in 96-well microtiter plates. A standard 5-hr ^{51}Cr release assay then was performed with 1×10^4 EL4 or EG7 cells as target cell lines at various effector to target ratios. The % specific lysis was calculated as: [(cpm of sample − cpm of spontaneous release)/(cpm of maximum release − cpm of spontaneous release)] × 100. Spontaneous release was defined as the mean cpm released from five replicates of 1×10^4 labeled cells incubated in media alone. Maximum release was defined as the mean cpm released from five replicates of 1×10^4 labeled cells incubated in media containing Triton X-100 (Sigma).

Detection of Lux Activity. Mice were injected with 100 μg of IE-lux i.m. into both quadriceps or i.d. at the base of the tail. Five days later, the entire quadriceps or dermis at the injection site were removed and frozen by placing on dry ice. Frozen tissues were then ground to a fine powder with a mortar and pestle and homogenized in a 1.5-ml microfuge tube with a hand-held homogenizer (PT 1200; Kinematica, Lucerne, Switzerland) in 1 ml of cell culture lysis reagent (Promega). Lux activity was determined with the luciferase assay system (Promega) according to the manufacturer's instructions. Standard curves were generated by using lux protein (L 9506; Sigma).

RESULTS

Ab Levels and Cytokine Responses. To determine whether the Ab response generated by DNA immunization was different from that obtained with protein immunization, mice were immunized with DNAs expressing OVA, HEL, and hIg and compared with varying doses of soluble protein. Mice were immunized on day 0, boosted on day 14, and bled 2 and 4 weeks post-initial immunization. The antigen-specific IgG and IgG subclass levels were determined by ELISA. Our initial experiments using lux as a reporter construct estimated the expression levels in i.m. and i.d. DNA-injected mice to be similar and in the nanogram range (data not shown). However, at least 1,000-fold more soluble protein was required to obtain similar total IgG responses for all three antigens tested. The route of administration (i.d. vs. i.m.) was compared for DNA and protein injections of OVA and hIg. I.d. immunization for both antigens produced higher IgG responses than equivalent doses of either DNA or protein via the i.m. route. The results obtained in one experiment with OVA are shown at 2 and 4 weeks (Fig. 1). These differences were most evident early (2 weeks) in the response although still significantly different at 4 weeks ($P < 0.05$, Student's t test). In numerous experiments, mice receiving empty vector or saline did not mount an OVA-specific Ab response.

The IgG subclass response to OVA and hIg was examined at 4 weeks, and the i.d. route was shown to elevate IgG1 levels for both protein (results obtained with OVA are shown; Fig. 2*A*) and DNA immunization ($P < 0.05$) (results obtained with OVA or hIg are shown; Fig. 2 *A* and *B*). Similar results to those shown were obtained in repeated experiments. Interestingly, i.d. DNA immunization more closely mimics the subclass

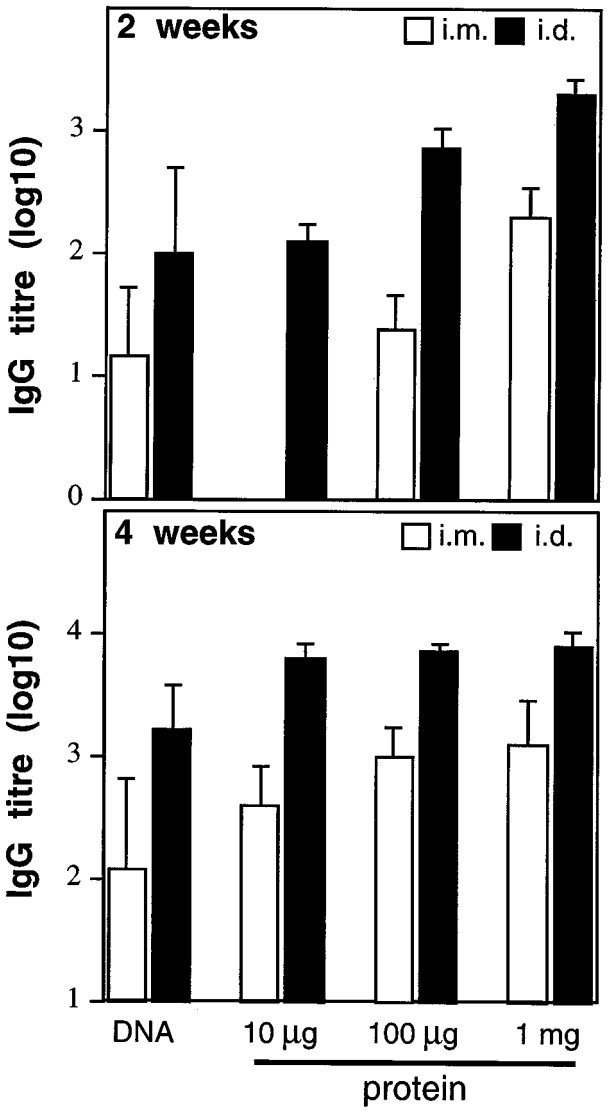

FIG. 1. Influence of the route of administration on the OVA-specific IgG responses. BALB/c mice were immunized i.m. or i.d. with 100 μg CIGH-OVA (DNA) or the indicated dose of OVA protein (protein) in normal saline. Sera were obtained at 2 and 4 weeks post-initial immunization (day 0) and stored at −20°C until assayed for OVA-specific IgG in an ELISA. Titers were defined as the highest dilution to give a 0.2 OD at 450 nm. Results are expressed as the mean of the log10 titer ± SEM from five mice in each group.

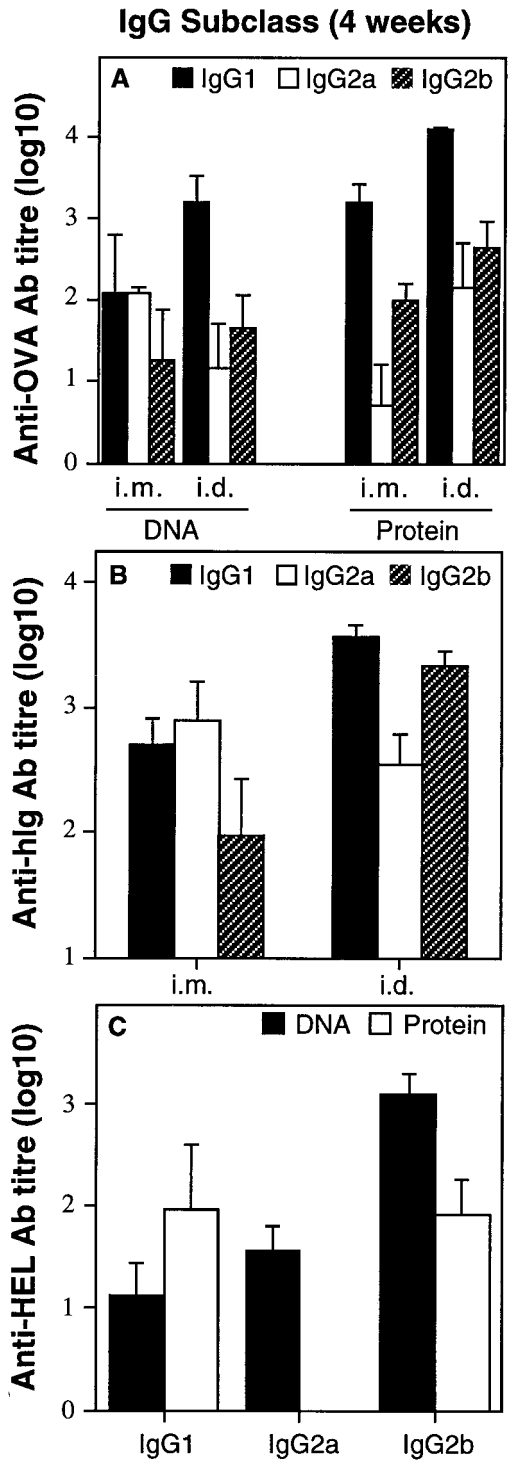

FIG. 2. Influence of the route of administration on the IgG subclass responses. At 4 weeks post-initial immunization sera were obtained and assayed for Ag-specific IgG1, IgG2a, or IgG2b in an ELISA. Titers were defined as the highest dilution to reach an OD of 0.2 at 450 nm. (*A*) BALB/c mice were immunized with 100 μg of CIGH-OVA (DNA) or 100 μg of OVA protein (protein) in normal saline. Results are expressed as the mean of the log10 titer ± SEM from five mice in each group immunized with CIGH-OVA or OVA protein. (*B*) BALB/c mice were immunized with 100 μg of hIg encoding DNA in normal saline i.m. or i.d. Results are expressed as the mean of the log10 titer ± SEM from six mice in each group. (*C*) BALB/c mice were immunized i.m. with 100 μg of CIGH-HEL (DNA) or 25 μg of HEL protein (protein) in normal saline. Results are expressed as the mean of the log10 titer ± SEM from five mice immunized with HEL protein and from 16 mice immunized with CIGH-HEL.

response to protein immunization with an IgG1 dominance than i.m. DNA immunization. I.m. DNA injection as compared with i.m. protein (with OVA) results in elevated IgG2a levels ($P < 0.05$; Fig. 2*A*). These differences were most evident with HEL and OVA ($P < 0.05$) and least evident with hIg immunization (did not reach statistical significance; data not shown); the subclass response to HEL protein was entirely IgG1 and IgG2b with no detectable IgG2a whereas the response in after i.m. DNA in all 16 mice tested was IgG2a/IgG2b dominated (Fig. 2*C*). Our overall conclusion is that immunization with i.m. DNA is IgG2a dominated, whereas i.m. or i.d. protein tends to be IgG1 dominated and i.d. DNA is somewhere in between. IgG switching to IgG1 or IgG2a has been linked to the actions of the cytokines IL-4 and INF-γ, respectively (12). We examined the IL-4 and INF-γ production from splenocytes *in vitro* after immunization with DNA or protein. IL-4 could be detected only after i.d. immunization, which enhanced IgG1 levels, with both protein and DNA (Table 1). INF-γ was elevated for both routes of DNA immunization although levels were more consistent between mice after i.m. immunization (similar to the IgG2a response). No cytokines could be detected when naive control splenocytes were stimulated or when splenocytes were cultured in the absence of antigen (data not shown).

Ab Avidity. The avidity of the Ab generated by DNA immunization was compared with soluble protein immunization (at a dose that gave comparable Ab levels) by either the i.m. or i.d. route. BALB/c mice, aged 6 to 8 weeks, were immunized with 100 μg of DNA expressing OVA or 100 μg of soluble protein in PBS on day 0 and 14. Mice were bled on day 28, and the avidity of the anti-OVA Ab was determined by calculating the concentration of soluble OVA required to inhibit the ELISA reactivity by 50% during the linear part of the response curve (I_{50}); the higher the I_{50} the lower the Ab avidity (10). The avidity of the Ab elicited by DNA immunization was 100- and 1,000-fold higher than that of protein immunization via the i.m. or i.d. route, respectively (Fig. 3). As with OVA, the avidity of the Ab response to HEL after i.m. DNA immunization was significantly higher ($P < 0.05$) than protein (data not shown). Interestingly, the avidity of the Ab produced by DNA immunization was similar to positive control sera from mice that were immunized with alum-precipitated OVA (data not shown). Route of immunization did not influence the avidity of the Ab response to soluble protein (Fig. 3) whereas the avidity appeared higher in i.d. compared with i.m. DNA-immunized mice (as seen in Fig. 3 although this difference between i.d. vs. i.m. DNA immunized mice did not reach statistical significance in all experiments). Because affinity maturation has been linked to the formation of germinal centers in lymphoid tissues (13–15) we investigated the possibility that the high-avidity Ab elicited after DNA immunization, as compared with soluble protein, could be caused by differences in germinal center formation. However, we were unable to detect germinal centers in frozen sections of spleen or lymph nodes from DNA- or soluble protein-

Table 1. Cytokine responses from immunized mice

		Cytokine*	
OVA form	Route	INFg (pg/ml)	IL-4 (pg/ml)
Protein	i.m.	387 ± 189	n.d.†
Protein	i.d.	389 ± 37	35 ± 6
DNA	i.m.	1,176 ± 372	n.d.†
DNA	i.d.	2,906 ± 1,946	37 ± 14

*Splenocytes (5×10^6/ml) were stimulated *in vitro* for 48 hr with OVA protein (1 mg/ml), and the cytokine levels were determined in the culture supernatant. The mean ± SD is shown for each group of three mice. No detectable cytokines were found in the culture supernatant that did not contain OVA protein.

†Not detectable.

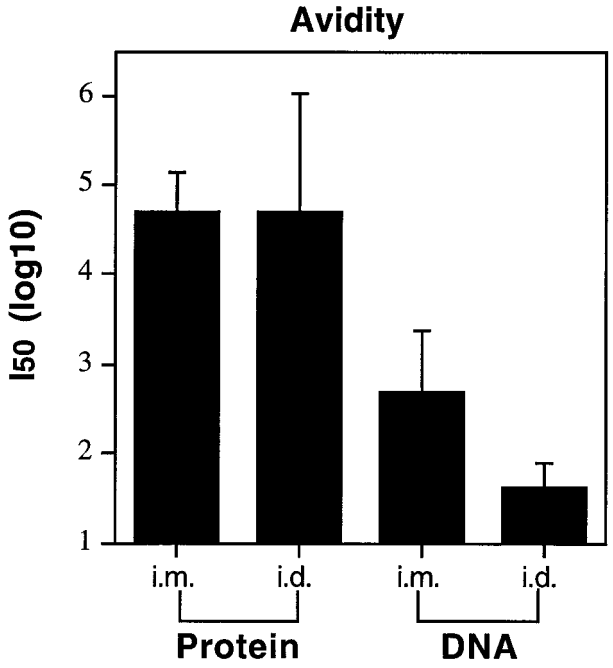

FIG. 3. Avidity of the antibody induced after DNA or protein immunization. Sera were obtained from BALB/c mice immunized with 100 μg of CIGH-OVA (DNA) or 100 μg of OVA protein (protein) in normal saline. The avidity is reported as the log of the concentration (10 mg/ml OVA was the highest concentration used) added to the well that resulted in a 50% binding inhibition of each immune sera control (I_{50}). Results shown are the mean $I_{50} \pm$ SEM from five mice in each group.

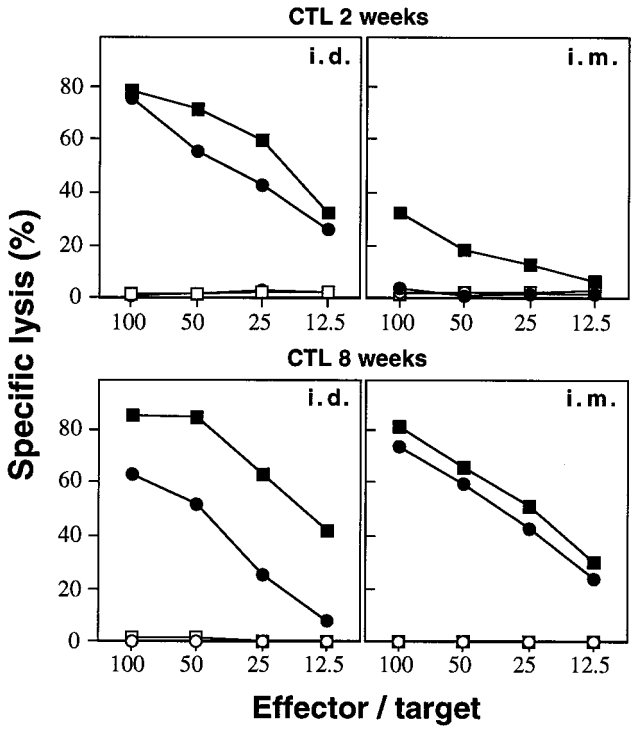

FIG. 4. Kinetics of the CTL response in DNA-immunized mice. Splenocytes from BALB/c × C57BL/6 mice were taken 2 or 8 weeks postimmunization with CIGH-OVA, restimulated *in vitro* for 5 days, and tested for their ability to lyse EG7 (solid symbols) or control EL4 (open symbols) in a standard ^{51}Cr release assay. Results shown are from two representative animals at each time point. No specific lysis of EG7 or EL4 cells was seen with naive control animals.

immunized mice (data not shown) possibly because of the low dose and lack of adjuvants such as alum used in our study.

CTL Responses. CTL responses to OVA were examined at 2 weeks (after a single immunization) or at 6 to 8 weeks (after 2 immunizations) in H2-K^b mice. CTL responses of two representative animals from several experiments are shown (Fig. 4). I.d. immunization gave the highest CTL responses early (2 weeks) (Fig. 4). In the vast majority of i.m. immunized mice, CTL responses could not be detected at 2 weeks (data not shown). However, after two immunizations and 8 weeks there was little difference in the CTL response between i.d. and i.m. DNA immunized mice (Fig. 4). As expected, splenocytes from mice immunized with saline, control vector, or soluble protein were not able to lyse the OVA-expressing cell line (data not shown). Direct transfection of APCs has been shown after biolistic DNA immunization (6) and proposed for i.d. immunization (2) and excluded from i.m. immunization (16). The possibility that the stronger CTL responses 2 weeks after i.d. immunization could be because of direct transfection of APCs was examined. The cytomegalovirus promoter and intron were replaced with the major histocompatibility complex IE alpha chain promoter that restricts expression to class II positive cells (17) and therefore protein should be expressed in APCs. The lux reporter cDNA was used such that protein produced *in vivo* could be detected. Mice were injected i.m. or i.d. with 100 μg of IE-lux DNA, and the relevant tissue was removed 5 days later for detection of lux activity. Significant lux activity could be detected, although at low levels, in skin samples from i.d. immunized mice whereas muscles from i.m. immunized mice did not contain lux activity above background (Fig. 5). This finding is consistent with the notion that i.d. but not i.m. DNA immunization can directly transfect class II positive cells (most likely professional APCs).

DISCUSSION

Some remarkable differences were evident when immune responses were compared between DNA- and soluble protein-immunized mice by using the antigens OVA, hIg, or HEL. We also investigated how the response may be influenced by dose and, in the case of OVA and hIg, route of immunization. The most striking difference observed was that the avidity of the Ab generated after DNA immunization was up to 1,000-fold higher than soluble protein immunization at doses that generated similar total IgG responses. The i.d. route was shown to produce higher Ab levels than i.m. injection for both protein and DNA immunization probably because of elevated IgG1. Protein immunization produced a IgG1 predominance regardless of route, whereas i.m. DNA immunization produced more IgG2a. Evidence for direct transfection and expression of protein by class II positive cells was obtained by demonstrating lux expression by using an IE alpha chain promoter in i.d. DNA-immunized mice only.

DNAs encoding secreted antigens were used because we previously had shown optimum responses when the antigen was secreted vs. cell associated (18) and they most closely mimic soluble protein immunization. However, DNA immunization was shown to enhance dramatically the avidity of the Ab as compared with soluble protein. As mentioned earlier, DNA immunization differs from soluble protein immunization in that the 1,000-fold higher doses of soluble protein than that elicited by DNA are required to generate similar Ab levels. It has been shown that affinity of Ab is not a direct function of dose (19) and argues against the high-avidity Ab elicited by DNA immunization to be attributable to the lower dose. However, high-affinity Ab, similar to that achieved with protein in adjuvants, can be generated by daily immunization with low doses of protein although Ab could be detected only in three of 10 mice (20). DNA immunization would be similar to this method given the low-dose continual secretion of antigen although seemingly much more effective in terms of seroconversion rates. Ab affinity has been directly correlated with

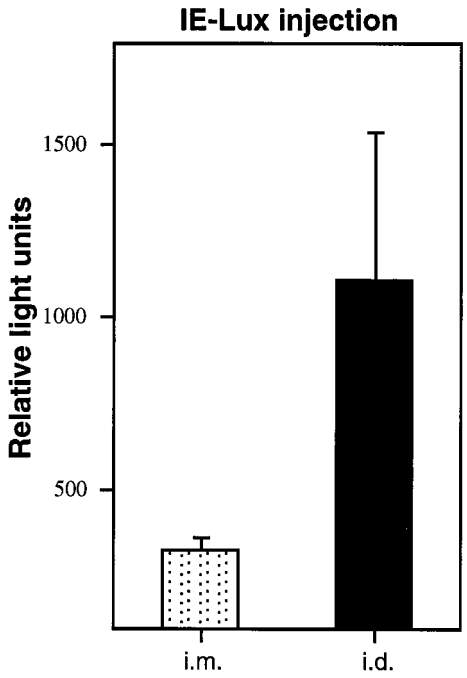

FIG. 5. Lux expression after injection of IE-lux DNA. Mice were injected with 100 μg of IE-lux i.m. into both quadriceps or i.d. at the base of the tail. The entire muscle or injected skin area was removed 5 days later to determine the lux activity. Results shown are the mean ± SEM relative light units from eight injected muscles and four skin samples. Background activity in uninjected tissues was 306 ± 28 for muscle and 377 ± 71 for skin.

immunological functions such as the ability to neutralize virus (21) and fix complement (22), both of which would influence vaccine efficacy. Therefore effects on antibody avidity should be considered when altering DNA immunization systems. In particular, reducing continuous antigen expression (and hence rendering it more similar to a single bolus of protein immunization) may be detrimental to Ab avidity and possibly overall vaccine efficacy.

Affinity maturation has been linked to the formation of organized structures within lymphoid tissues known as germinal centers. We hypothesized that DNA immunization could enhance the formation of germinal centers and thereby the affinity of the Ab generated. However, we were unable to detect germinal center formation in either DNA- or soluble protein-immunized mice. Because germinal centers can be readily detected in mice receiving protein with adjuvant (23) overt germinal center formation may require an immunological challenge of much greater magnitude than DNA or soluble protein immunization. It is interesting to note that INF-γ has been shown to increase the affinity of Ab to a T cell-dependent antigen (24), and we showed that splenocytes from DNA-immunized mice secreted higher levels of this cytokine after *in vitro* restimulation. On the other hand, the higher avidity Ab induced after DNA immunization may have occurred in the absence of high-frequency somatic hypermutation associated with germinal centers. It has been shown previously that germ-line selection of B cells can occur before hypermutation (25). The low level of antigen secreted via DNA immunization could have served to select B cells with high avidity for antigen. Thus, B cells expressing germ-line high-avidity Ab would have a major advantage to compete for the low level of antigen, gain T cell stimulation, and expand. In contrast, bolus protein injection would provide antigen that was readily available for all B cells including those of low affinity.

Elevated IgG titers were found in mice immunized with DNA or protein via the i.d. route for both OVA and hIg. IgG1 was elevated after i.d. immunization and may account for this difference in total Ab levels. DNA immunization via the i.m. route yielded higher IgG2a levels than protein for OVA and most dramatically with HEL. Higher Ab responses after i.d. DNA immunization also have been reported (2), although the doses compared in this study were lower than optimal for i.m. DNA immunization (26). Subsequently, Raz *et al.* (27) compared the response to i.d. protein vs. i.d. DNA encoding the cytoplasmic antigen β-galactosidase. They found that i.d. DNA induced a predominantly IgG2a response (whereas protein, as with our study, was predominantly IgG1). The discrepancy to our study may be attributable to their use of an antigen localized within the transfected cell vs. our use of secreted antigens, which would more closely mimic soluble protein injection (and hence a more valid comparison). There also may be variation in the subclass dominance because of antigen given that we found a strong IgG1 predominance to soluble OVA and HEL but not as strong with hIg immunization. An IgG2a predominance after i.d. DNA immunization with influenza hemagglutinin also has been reported, but unfortunately a comparison with protein immunization was not performed (28).

We found that there was an elevated CTL response at 2 weeks in mice immunized i.d. with DNA. However, i.d. vs. i.m. responses were similar at 8 weeks. Furthermore, a large proportion of the mice immunized i.m. failed to mount a CTL response that was detectable at 2 weeks. The mechanism of CTL priming after i.m. DNA immunization has recently been investigated (16, 29). One report (16) showed that severe combined immunodeficient mice of the H-2^b or ^{d} haplotype infused with $b \times d$ F_1 splenocytes generated CTLs against the DNA-encoded antigen that was restricted by the severe combined immunodeficient host haplotype. However, if these mice also received $b \times d$ bone marrow, then CTLs of both *b* and *d* haplotypes were generated, indicating that this cross-priming was dependent on presentation of antigen by a bone marrow-derived cell. These results were obtained even when mice were immunized 21 days before bone marrow and spleen cell transfers, thereby ruling out that this result could be caused by direct transfection of APCs after i.m. immunization. As mentioned earlier, direct transfection of APCs has been shown after biolistic DNA immunization (6) and proposed for i.d. immunization (2). This finding is in agreement with our results using the IE alpha chain promoter, which showed expression of lux after i.d. but not i.m. DNA immunization and would argue that direct transfection of APCs did occur. The expression of an antigen in an APC capable of directly priming a CTL response after i.d. immunization is in contrast to the cross-priming mechanism of i.m. immunization (whereby the antigen is transferred from the myocyte to an APC). This expression may be one reason to account for the more rapid induction of CTL priming observed after i.d. immunization.

This study represents a comprehensive comparison of DNA vs. protein immunization via two possible routes of immunization. The use of three antigens, DNA-encoding secreted antigens (which most closely mimic soluble protein immunization) and doses of protein that gave similar total Ab responses, allowed us to determine which of the differences observed were because of route, antigen, dose, or the uniqueness of DNA immunization. The higher Ab levels, the Ab subclass differences, and more rapid CTL priming observed argue that the mechanisms of priming after i.d. DNA immunization differs from i.m. Similarly, the higher Ab avidity, different Ab subclass predominance, and the ability to induce CTLs would argue that the priming of both B and T cells may be different for DNA vs. protein immunization. Therefore methods such as coinjection of cytokines that affect the response in one route may not be useful for the other or that affect protein may not affect DNA immunization. It recently has been shown that there is a threshold of avidity required for

Ab-mediated protection to occur (30). Given that alum-based vaccines have been effective in humans and that DNA can induce Abs of comparable avidity, this finding would suggest that DNA vaccines would achieve the effective threshold. Thus, DNA immunization offers two advantages over soluble protein immunization for vaccination, the generation of CTL responses and high-avidity Ab, both of which are induced in the absence of adjuvant.

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