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# Measles Virus DNA Vaccination: Antibody Isotype Is Determined by the Method of Immunization and by the Nature of both the Antigen and the Coimmunized Antigen

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**Plasmids encoding the measles virus hemagglutinin (HA) and nucleoprotein (NP) proteins inoculated into the skin of BALB/c mice by the gene gun method induced both humoral and cytotoxic lymphocyte class I-restricted immune responses. Although intramuscular immunization induces the immunoglobulin G2a (IgG2a) antibody isotype for both antigens, with gene gun immunization, the NP still generated mainly IgG2a and the major isotype induced by the HA was IgG1. Interestingly, gene gun coimmunization of HA and NP plasmids resulted in a dominant IgG1 HA response and the switching of antibodies generated against the NP to the IgG1 isotype.**

The initial studies showing that injection of DNA into muscle induces an immune response to the encoded protein opened a new approach to vaccination (for reviews, see references 19 and 22). Recent studies suggest that inoculated muscle cells probably act only as a source of antigen and that immune priming takes place elsewhere in the body (14). For example, excision of an injected muscle a few minutes after DNA inoculation did not affect antibody and cytotoxic T-lymphocyte (CTL) responses (21). Thus, it may be interesting to examine other DNA delivery systems to study how the immune system responds to DNA vaccination. One alternative system involves precipitating DNA onto gold beads which are then propelled into the skin by means of pressurized helium gas (12). When such a system is used, less DNA is required, but unlike the case with intramuscular inoculations, the response is Th2-like, generating immunoglobulin G1 (IgG1) antibodies (17). More recent observations suggest that this is probably due to the mode of inoculation rather than the route (10).

We have been studying DNA vaccination against the paramyxovirus measles virus (MV). This disease is one of the primary causes of infant mortality in developing countries, and there is an urgent need for an effective vaccine in infants, as the present live attenuated vaccine is inefficient in the presence of maternal antibodies. Our previous studies established that in a mouse model at least three MV proteins play a role in protection (23). Both glycoproteins, hemagglutinin (HA) and fusion, induce neutralizing antibodies (9, 11), and HA and nucleoprotein (NP) induce CTLs (3, 4), which do not protect against infection but help in recovery (5). In our previous study on DNA vaccination, we showed that intramuscular inoculation of DNAs coding for the MV HA and NP (pV1J-HA and pV1J-NP [6]) induced class I-restricted CTLs and a humoral response corresponding to a Th1 response (6). In the present study, we have extended our observations to compare the same plasmids' ability to induce an immune response when they are delivered into the skin by a gene gun (Bio-Rad, Ivry sur Seine, France). Gold beads were coated with DNA as follows: approximately 30 mg of gold powder (1.0-μm gold beads; Bio-Rad) was mixed with 100 μl of 0.1 M spermidine (Sigma, L'Isle D'Abeau, France). After sonication, 0.5, 2, or 5 μg of plasmid DNA was added per mg of gold powder, and then 200 μl of 2.5 M $CaCl_2$ was added to the mixture, with gentle vortexing. Pellets were washed three times and suspended in cold 100% ethanol. Tubes containing dried DNA-coated gold beads were stored at 4°C.

**Immune response to MV HA DNA.** Six- to eight-week-old female BALB/c mice (Iffa-Credo, Domaine des Oncins, France) were immunized via the shaved abdominal epidermis one to three times at 21-day intervals with 0.5, 2, or 5 μg of pV1J-HA DNA/mg of gold beads. Two gene gun inoculations (each containing 0.5 mg of gold beads) were given for each dose. The antibody levels measured by enzyme-linked immunosorbent assay, as previously described (6), reached a plateau after two inoculations and did not significantly increase with a third inoculation (result not shown).

Our previous studies with intramuscular inoculation established that pV1J-HA induced IgG2a antibodies which are

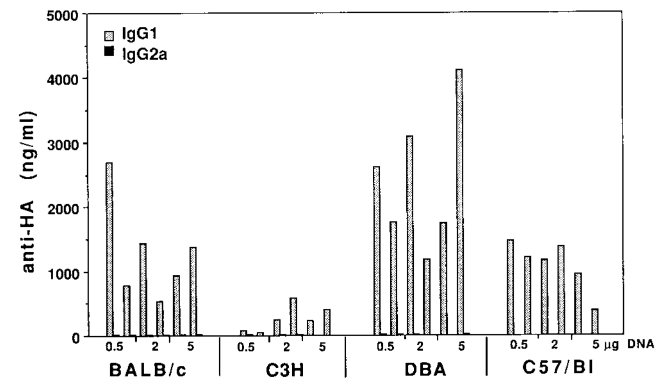


FIG. 1. Anti-MV HA isotype of antibodies induced in BALB/c, DBA/2 (*H-2^d^*), C3H (*H-2^k^*), and C57/Black (*H-2^b^*) mice immunized with 0.5, 2, or 5 μg of pV1J-HA by epidermal gene gun. Sera were collected 3 weeks after the immunization. Sera from mice immunized with a control pV1J had means ± standard deviations of 158 ± 198 ng/ml for IgG1 anti-HA antibodies ($n = 11$) and 10 ± 18 ng/ml for IgG2a anti-HA antibodies ($n = 11$). Data represent individual animals.

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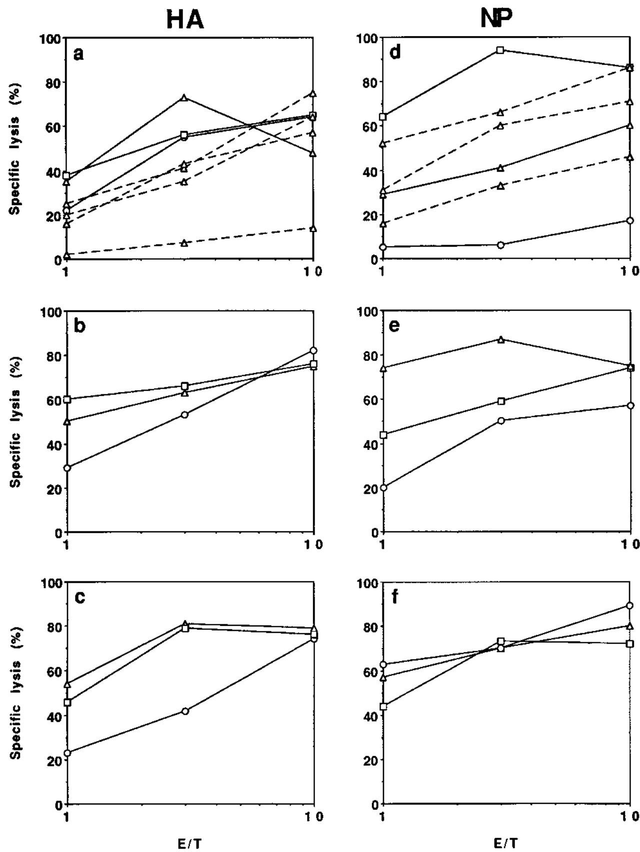


FIG. 2. Anti-MV HA and NP CTL response after immunization with pV1J-HA or -NP, respectively. BALB/c mice were immunized with 0.5 (circle), 2 (triangle), or 5 (square) μg of pV1J-HA by epidermal gene gun one (a, d), two (b, e), or three (c, f) times at 3-week intervals. The spleen cells were removed 3 weeks (continuous line) or 8 days (dotted line) after the last immunization. After in vitro stimulation with P815-HA or -NP cells, respectively, lysis was measured on P815-HA or -NP cells, and P815 cells were used as a negative control. The results show the specific lysis of targets at graded effector/target ratios. Each curve represents an individual animal.

associated with a Th1-type response. When we studied the antibody isotype induced in BALB/c by the gene gun immunization, we observed that it was mainly IgG1 (Fig. 1). These data are similar to those described for influenza hemagglutinin by Feltquate et al. (10). The antibody isotype did not vary with time after immunization, number of immunizations, or the amount of plasmid used (data not shown) and was not influenced by genetic background, as pV1J-HA-immunized DBA/2 (*H-2*$^{d}$), C3H (*H-2*$^{k}$), and C57/Black (*H-2*$^{b}$) mice induced mainly the IgG1 isotype (Fig. 1).

To study CTL activity, spleen cells from the immunized mice were stimulated in vitro and analyzed in a cytolytic assay as previously described (6). Despite the apparent Th2-type response, good memory CTL responses were obtained with all protocols used, even when responses were measured just 8 days after a single immunization (Fig. 2), and persisted for several months.

**Immune response to MV NP DNA.** BALB/c mice were immunized with pV1J-NP with the gene gun and a similar schedule of immunizations. The antibody response with the different number of doses and different plasmid concentrations was similar to that observed for HA, i.e., increased levels after one boost. Similar antibody levels were induced in the range of 0.5 to 5 μg of DNA (data not shown). As was previously shown by intramuscular inoculation (6), the antibody isotype induced was mainly IgG2a (Fig. 3), in contrast to the HA results. One explanation for this could be that as the NP is a cytosolic protein and the HA is membrane bound, the potential processing and presentation of the two proteins may be different. However, the same argument would be valid for intramuscular inoculation. Furthermore, it has been reported that gene gun immunization with influenza NP induces a Th2 response (17), so clearly the directed differentiation of T cells is more complicated than a simple distinction between cytosol and membrane-bound proteins. The two methods of immunization (intramuscular versus gene gun) target different cell types, possibly influencing the T-cell response. Furthermore, 9 weeks after immunization, one-third of the 18 mice analyzed showed increased levels of anti-NP IgG1 over IgG2a, regardless of the quantity of DNA injected or the number of inoculations (data not shown). CTL responses were also high, even after a single inoculation (Fig. 2).

**Coimmunization of HA and NP DNA.** Our results show that when injected by the gene gun, the different MV proteins induce different antibody isotypes. This phenomenon has been suggested to parallel the induction of Th1 and Th2 pathways (1). The pathway taken has been shown to be influenced by the induction of certain cytokines. To determine if coimmunization of these two plasmids would influence the isotype of the antibody response, BALB/c mice were immunized with a mixture of pV1J-HA and pV1J-NP in ratios of 1:1, 4:1, or 1:4 while the total amount of DNA was kept constant (5 μg).

Measurement of the anti-HA isotype antibody in mice vaccinated with the different mixtures showed it to be mainly IgG1, similar to that for HA alone (data not shown). In contrast, the anti-NP antibodies switched from the IgG2a to the IgG1 isotype after coimmunization (Fig. 4). The proximity of expression of the two antigens was not important in this switching effect, as when pV1J-HA and -NP were inoculated separately in different areas of the skin, the antibody response induced 3 weeks later was the same as that induced when the mixture was inoculated (Fig. 4). When analyzed 6 weeks later, only one of six mice showed IgG2a predominance.

Cytokines have been used to direct the immune response in several studies. Expression of interleukin-12 either alone or

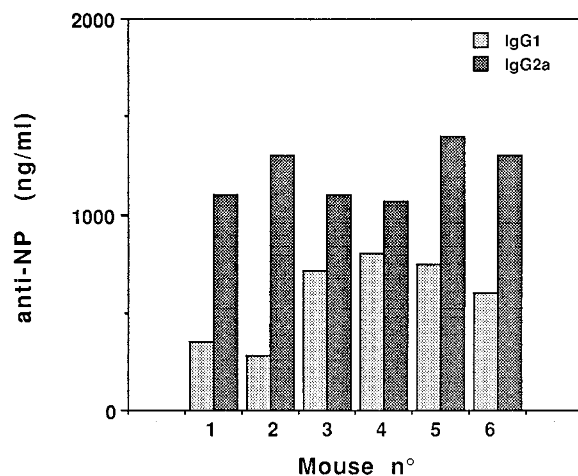


FIG. 3. Anti-MV NP antibody response as measured by enzyme-linked immunosorbent assay in BALB/c mice immunized with 5 μg of pV1J-NP by epidermal gene gun. Sera were collected 3 weeks after immunization. Each pair of bars represents an individual animal. Sera from mice immunized with a control pV1J had means ± standard deviations of 13 ± 45 ng/ml of IgG1 anti-NP antibodies ($n$ = 11) and 83 ± 276 ng/ml of IgG2a anti-NP antibodies ($n$ = 11).

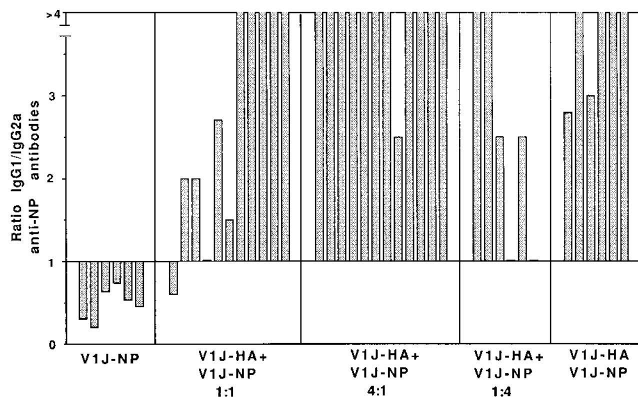


FIG. 4. Relationship between the isotype of anti-NP antibodies in sera from mice immunized with 5 μg of pV1J-NP or mixtures of pV1J-HA and pV1J-NP at ratios of 1:1, 4:1, and 1:4 so that the total quantity of DNA/mg gold beads was 5 μg, or pV1J-HA and pV1J-NP injected in different skin area. BALB/c mice were immunized by epidermal gene gun. Sera were collected 3 weeks after immunization. Data are results for individual animals.

with immunizing antigens can increase protection against microbial pathogens (2) or tumors in animal models (13, 18), in parallel with a Th1 response. Expression or addition of interleukin-4 with the immunogen induces a Th2 response (16, 20). The local concentrations of the cytokines in the initial priming of the immune response are probably critical, as once the T cells have been committed, they cannot be modified. Although some studies have suggested the possibility of Th1 and Th2 switching, a more recent study has shown that once differentiated, T cells cannot switch (15). In agreement with this, Feltquate et al. (10) have shown that initial immunization establishes the Th-cell type of the immune response and that this is not modified by subsequent alternative methods of immunization.

Acute viral infections induce a Th1 response, whereas soluble proteins favor a Th2 response (7). When tetanus toxoid was administered 1 day after viral infection, the response to this soluble protein changed from Th2 to Th1 (8). Presumably, this change is due to the domination by the cytokines induced by the viral infection of those produced by the tetanus toxoid. In our studies, we observed that after the coexpression of MV HA and NP, the HA-induced Th2 response was dominant. These observations will obviously have an impact on DNA vaccination, as DNAs coding for several pathogens should ideally be administered concomitantly.

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