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Gene gun bombardment with gold particles displays a particular Th2-promoting signal that over-rules the Th1-inducing effect of immunostimulatory CpG motifs in DNA vaccines

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Abstract

The mode of administering a DNA vaccine can influence the type of immune response induced by the vaccine. For instance, application of a DNA vaccine by gene gun typically induces a Th2-type reaction, whereas needle inoculation triggers a Th1 response. It has been proposed that the approximately 100-fold difference in the amount of DNA administered by these two methods is the critical factor determining whether a Th1 or a Th2 response is made. To test this hypothesis, BALB/c mice were immunized with two plasmid DNA constructs encoding different proteins (OspC/ZS7 of *Borrelia burgdorferi* and Bet v 1a, the major birch pollen allergen). Both vaccines were applied by needle and/or by gene gun immunization at the same and at different sites of injection. An analysis of the IgG subclass distribution and measurement of IFN-γ after antigen-specific lymphoproliferation does not support the widely accepted view that Th2-type immunity induced by gene gun application is solely due to the low amount of injected plasmid DNA thus falling below the critical concentration of CpG motifs necessary for Th1-induction. Furthermore, the data also indicate a strong and even systemic adjuvant effect of the gene gun shot itself.

Keywords: DNA immunization; Gene gun; Immunomodulation

1. Introduction

Since the first publications on the use of “naked” plasmid DNA as a tool for immunization, experimental DNA vaccines able to induce humoral and cellular immune responses have been developed for a wide variety of antigens and in many cases it has been possible to show protection against infection with viral, bacterial and parasitic pathogens [1]. DNA-based vaccination has become an attractive alternative to conventional immunization strategies, particularly to those approaches using attenuated live pathogens as immunogens.

DNA vaccines are usually administered by intradermal (i.d.) or intramuscular (i.m.) injection or DNA-coated gold particles are propelled into the epidermis using a gene gun. Both needle and gene gun DNA delivery have been shown to induce cellular as well as humoral immunity in several antigen systems [2–6]. However, the method of antigen delivery can affect the type of immune response made. Typically, i.d. and i.m. injections induce a predominant Th1-type response with elevated IgG2a levels and reduced IgG1 levels, whereas DNA applied using a gene gun predominantly produces IgG1. These differences between needle injection and gene gun inoculation were attributed to the amount of plasmid DNA, and associated CpG motifs, since needle injection requires approximately 100-fold more DNA than gene gun immunization to generate an equivalent antibody response [3,5–8]. On the other hand, differences in transfection efficacy of antigen presenting cells and the nature of the antigen itself may also contribute to the T helper cell bias that is ultimately generated. Whereas dendritic cells (DC) can be directly transfected through gun immunization [9], they do not readily take up extracellular DNA [10]. Therefore, it is assumed that plasmid DNA administered by needle injection is taken up preferentially by keratinocytes, which produce the antigen and transfer it to Langerhans cells of the skin [11]. Furthermore, the localization of the expressed antigen

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(membrane-bound, cytosolic, or secreted) can influence the potential for uptake, processing and presentation, thereby also influencing the T helper profiles [12–19].

In the present study, we critically addressed the hypothesis that only the injected amount of DNA (correlating with the amount of CpG motifs) is responsible for the different immune response types induced by needle injection of saline DNA or gene gun application of DNA precipitated onto gold beads [7,8].

For this purpose, we applied two different antigens (the outer surface protein C from *Borrelia burgdorferi* strain ZS7, and Bet v 1a, the major birch pollen allergen) by i.d. saline needle injection or epidermal gene gun inoculation. We have previously shown that both of these antigens typically raise Th1-type immune responses after i.d. needle injection and Th2-type reactions following gene gun application [20–22].

The constructs encoding these two antigens were co-immunized either via the same method (i.d. or gene gun) or different methods (i.d. and gene gun) into the backs or to the abdomens of BALB/c mice. Our results show that the immune reactions induced by gene gun or needle injection against different antigens are not independent of each other. In contradiction to the hitherto widely accepted assumption, the data elicited that the "danger" signal induced by gene gun immunization dominated over the DNA-induced Th1 signal mediated by CpG motifs.

2. Materials and methods

2.1. Plasmids used for immunization

Construction of the plasmids pCMV-OspC encoding OspC [20] and pCMV-Bet encoding Bet v 1a [23] was described earlier.

Endotoxin content of the plasmids was less than 50 ng LPS/mg DNA, as measured by Limulus amoebocyte assay (Pyroquant, Walldorf, Germany).

2.2. Animals and immunization protocol

Mice used for immunizations were 6–8-week-old BALB/c females from Himberg (Austria). Sera were collected before the first immunization and at regular intervals thereafter. Sera were preserved by adding sodium azide (final concentration of 0.2%) and stored at 4 °C. Mice were vaccinated four times at 3-week intervals with pCMV-OspC and/or pCMV-Bet by i.d. saline needle injection, e.d. using a Helios gene gun (Bio-Rad, Hercules, CA), or a combination of both (Table 1). Animals of the control experiment that received gold particles lacking plasmid DNA were immunized five times in 10-day intervals with pCMV-OspC by i.d. saline needle injection.

Plasmid DNA was precipitated onto gold beads (1.6 μm diameter) by $CaCl_2$ in the presence of spermidine at a loading rate of 2 μg DNA/mg of gold. Mice received a total of 1 μg of DNA per construct. For i.d. immunization, 50 μg of the appropriate plasmid DNA was applied in 50 μl PBS into one spot. Animals of the separate control experiment received 100 μg plasmid DNA in 100 μl PBS injected into two sites following particle bombardment.

2.3. Analysis of antibody subclasses

Two weeks after the final immunization sera were analysed for IgG1 and IgG2a antibodies. Black 96-well high-bind immunoplates (Greiner, Kremsmuenster, Austria) were coated by overnight incubation at 4 °C with recombinant antigen at a concentration of 1 μg/ml in PBS.

Table 1
Co-immunizations with heterologous antigens and the resulting IgG1:IgG2a ratios[a]

Groups	Method/site[b]				Injection[c]	IgG1:IgG2a	
	pCMV-Bet		pCMV-OspC			Bet v 1a	OspC
A	Needle	Back	Gun	Abdomen	–	1.5	29.7
B	Gun	Abdomen	Needle	Back	–	3.1	7.5
C	Gun	Abdomen	Gun	Abdomen	Applied to different spots	2.4	52.8
D	Needle	Back	Needle	Back	Applied to different spots	0.2	1.3
E	Needle	Abdomen	Gun	Abdomen	Needle injection into gun spot	3.3	62.2
F	Gun	Abdomen	Needle	Abdomen	Needle injection into gun spot	4.5	15.4
G	Needle	Back	Needle	Back	Applied to the same spot	0.1	0.1
H	Gun	Abdomen	Gun	Abdomen	Applied to the same spot	1.6	118.0
Co1	–	–	Needle	Back	–	–	1.6
Co2	–	–	Gun	Abdomen	–	–	227.8
Co3	Needle	Back	–	–	–	0.02	–
Co4	Gun	Abdomen	–	–	–	3.9	–
Co5	–	–	Needle	Abdomen	–	–	0.3
Co6	Needle	Abdomen	–	–	–	0.1	–

[a] Mice were immunized four times at 3-week intervals with 50 (needle) or 1 μg (gun) of plasmid DNA coding for Bet v 1a or OspC. Gene gun bombardment was generally applied to the abdomen. IgG1 and IgG2a titers of sera taken 2 weeks after the final immunization were determined by ELISA. The IgG1:IgG2a ratios were calculated for each group ($n = 5$ for groups A–H; $n = 3$ for groups C1–C6).

[b] Each plasmid was either applied by i.d. needle injection or by gene gun. Immunizations were given to the shaved back or abdomen.

[c] When administering both plasmids to the abdomen/back, the plasmids were either given to the same spot, or to different spots.

Plates were washed with PBS/0.1% Tween 20 using the 96PW automatic ELISA-plate washing device (Tecan, Salzburg, Austria) and blocked with blocking buffer (PBS, 0.1% Tween 20, 0.5% BSA, pH 7.5) for 1 h at room temperature. Sera were serially diluted in blocking buffer, transferred to the coated microtiter plates, incubated for 1 h at room temperature and then washed. Horseradish peroxidase-conjugated rat anti-mouse IgG2a (PharMingen, San Diego, CA) or rat anti-mouse IgG1 (Serotec, Oxford, UK) detection antibody was added in blocking buffer (1:1000) and incubated for 1 h at room temperature. The luminometric assay was developed with Luminol (BM chemiluminescence substrate, Boehringer-Mannheim, Germany) diluted 1:2 in H_2O. Chemiluminescence (photon counts/second) was determined using a Lucy I Elisa-plate Luminometer (Anthos Labtec, Salzburg, Austria).

For end-point titer determinations any well with a luminescence greater than 3 S.D. above background (calculated using >20 wells containing no primary Ab) was scored as positive. The end-point titer of serum Ab bound to the plates was determined by using a standard curve generated with known dilutions of high titered antisera.

2.4. Quantification of IFN-γ and IL-4 in supernatants of re-stimulated splenocytes

Splenocytes were prepared 2 weeks after the final immunization, re-suspended in DMEM supplemented with 100 U/ml penicillin/streptomycin, 1% heat-inactivated FCS, 2×10^{-6} M 2-Me, 1 mM sodium pyruvate and 2 mM L-glutamine, and were distributed into 96-well, flat-bottom tissue culture plates (Beckton Dickinson, Franklin Lakes, NJ) at a density of 1×10^6 cells per well. Wells were treated with 20 μg/ml recombinant antigen. Five replicate wells were stimulated with each antigen for 72 h at 37 °C, 95% relative humidity and 7.5% CO_2.

IFN-γ and IL-4 in supernatants was quantified by sandwich ELISA using the OptEIA™ system (PharMingen, San Diego, CA). Briefly, cytokine was captured with monoclonal anti-mouse IFN-γ/IL-4 antibodies. Cytokine was detected by adding biotinylated anti-IFN-γ/IL-4 followed by avidin-conjugated HRP. The luminometric assay as described earlier was used for detection and IFN-γ/IL-4 was quantified by extrapolation from a standard curve prepared with recombinant murine IFN-γ/IL-4 (PharMingen, San Diego, CA).

2.5. Statistical analysis

Data are expressed as mean ± S.E.M. Statistical significance was assessed by an unpaired Student's *t*-test. For statistical analysis of subclass distributions, titers of individual mice were normalized (IgG1/(IgG1 + IgG2a)) and significant differences between groups were evaluated using a Mann–Whitney Rank Sum test. All calculations were performed with Sigma Stat for Windows version 2.0.

3. Results

Two weeks after the final immunization seroconversion was 100% in all immunization groups. At this time point, sera of individual mice from all groups were analysed for IgG1 and IgG2a. The IgG1:IgG2a ratios were taken as an indication of the type of T-helper immune response made. Immunization with plasmid DNA encoding Bet v 1a alone induced no antibody reaction against OspC and vice versa, pointing to a highly antigen-specific immune reaction (data not shown). An overview of all immunization schedules and the ratios of antigen-specific IgG1:IgG2a obtained is given in Table 1.

3.1. Needle immunization induces low IgG1:IgG2a ratios compared to gene gun immunization

Control mice that received plasmid DNA encoding OspC by i.d. injection into the back (Co1) or the abdomen (Co5) showed similar levels of OspC specific IgG1 and IgG2a (i.e. IgG1:IgG2a ratios of 1.6 and 0.3), indicative of a Th1-type helper response. Injecting both plasmid constructs into separate spots on the back (D), or as a mixture into a single spot on the back (G), also resulted in the induction of Th1-type helper cells (i.e. IgG1:IgG2a ratios of 1.3 and 0.1). The simultaneous administration of plasmid DNA encoding Bet v 1a resulted in a substantial increase in the magnitude of antibody response to OspC in comparison to the results obtained with the OspC plasmid alone (Fig. 1).

In the equivalent experiments conducted with the plasmid DNA encoding Bet v 1a, an even more pronounced Th1-type response was observed (Fig. 2). Control mice immunized into the back (Co3) or into the abdomen (Co6) had IgG1:IgG2a ratios of 0.02 and 0.1, respectively. Co-injecting OspC plasmid DNA constructs into separate spots on the back (D), or as a mixture into a single spot on the back (G), resulted in IgG1:IgG2a ratios of 0.2 and 0.1, respectively. The mean antibody titer for the control mice, which received an i.d. injection into the abdomen (Co6) was markedly

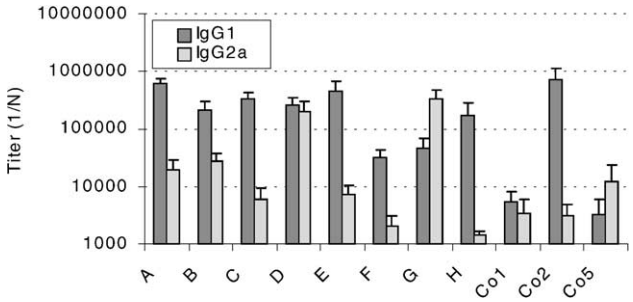

Fig. 1. OspC specific antibody subclass distribution measured 2 weeks after the fourth immunization. IgG1 and IgG2a titers of individual sera were determined by ELISA. The results are presented as mean ± S.E.M of titers for each group ($n = 5$ groups A–H; $n = 3$ groups Co1–Co5). See Table 1 for description of individual groups.

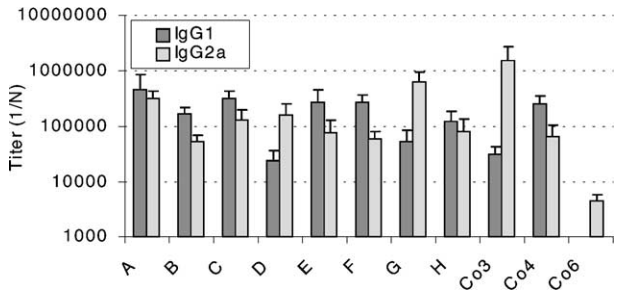

Fig. 2. Bet v 1a specific antibody subclass distribution measured 2 weeks after the fourth immunization. IgG1 and IgG2a titers of individual sera were determined by ELISA. The results are presented as mean ± S.E.M. of titers for each group ($n = 5$ groups A–H; $n = 3$ groups Co3–Co6). See Table 1 for description of individual groups.

lower than in the other groups (Co3, D and G) tested (Fig. 2).

As expected, control mice that received plasmid DNA by gene gun showed typical Th2-type responses. However, OspC (Co2) induced a more polarized Th2-type response than Bet v 1a (Co4) with an IgG1:IgG2a ratio of 227.8:1 compared to 3.9:1 for Bet v 1a. Gene gun immunization with both constructs to non-overlapping areas (group C) or to the same spot on the abdomen (group H) gave similar results to that observed using a single plasmid, i.e. IgG1:IgG2a ratios of 52.8 (C) and 118.0 (H) for OspC, and 2.4 (C) and 1.6 (H) for Bet v 1a (Figs. 1 and 2).

3.2. Gene gun immunization modulates the IgG1:IgG2a ratio of an independent immune reaction

It has been proposed that the difference in the T-helper profiles observed after i.d. and gene gun DNA immunizations is a consequence of the large differences in the amount of DNA, and DNA-associated CpG motifs, used in these two procedures. To test this hypothesis, groups of mice received one DNA vaccine applied by gene gun to the abdomen in 1 μg amounts and a second DNA vaccine by i.d. injection in 50 μg amounts into the back. The IgG1:IgG2a ratio of the immune response against the antigen, which had been administered by gene gun was pointing to a predominant Th2-type reaction as indicated by the typical Th2-type ratios, i.e. IgG1:IgG2a ratios of 29.7 (A) and 227.8 (Co2) for OspC, and 3.1 (B) and 3.9 (Co4) for Bet v 1a (Figs. 1 and 2). Surprisingly, in these co-immunization experiments, the type of immune response against the antigen given by i.d. needle injection was modulated towards a Th2-type i.e. IgG1:IgG2a ratios of 7.5 (B) compared to 1.6/0.3 (Co1/Co5, $P < 0.05/P < 0.05$) for a single needle administration of OspC, and 1.5 (A) compared to 0.02/0.1 (Co3/Co6, $P < 0.05/P = 0.07$) for a single needle administration of Bet v 1a (Figs. 1 and 2). This effect was slightly more pronounced when the needle injection and gene gun inoculation were given into the same spot, i.e. IgG1:IgG2a ratios of 15.4 (F) compared to 1.6/0.3 (Co1/Co5, $P < 0.05/P < 0.05$) for a single needle administration of OspC, and 3.3 (E) compared to 0.02/0.1 (Co3/Co6, $P = 0.07/P = 0.07$) for a single needle administration of Bet v 1a (Figs. 1 and 2). In this case, the IgG1:IgG2a subclass distribution concerning the antigen injected by needle resembled characteristic Th2-type ratios, i.e. IgG1:IgG2a ratios of 15.4 (F) compared to 227.8 (Co2) for a single gene gun administration of OspC, and 3.3 (E) compared to 3.9 (Co4) for a single gene gun administration of Bet v 1a (Figs. 1 and 2). A separate control experiment (Fig. 3A) demonstrated that this Th2-modulating effect of the gene gun was independent of the DNA vaccine but could also be induced by uncoated gold particles alone provided they were applied to the i.d.

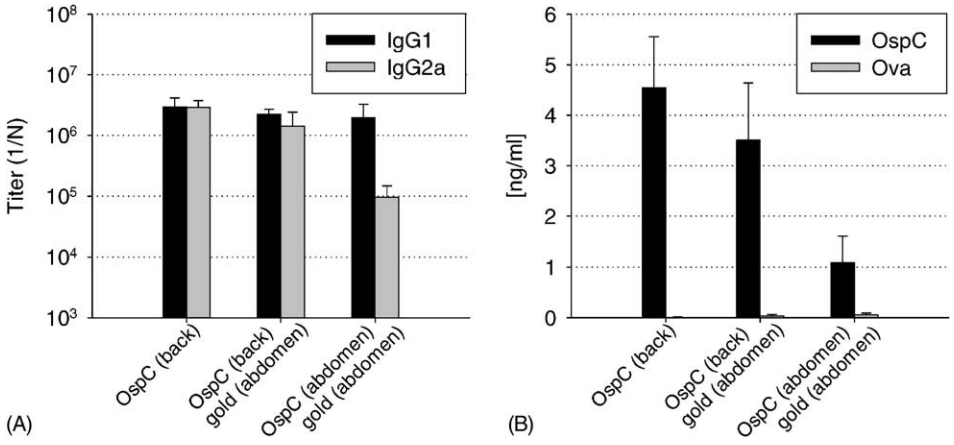

Fig. 3. Influence of co-application of gold particles lacking plasmid DNA together with i.d. saline needle injection. Mice were immunized five times at 10-day intervals with 100 μg of plasmid DNA encoding OspC. Prior to plasmid injection, gold beads lacking plasmid DNA were delivered to the shaved abdomen by two shots with a gene gun. Then, the plasmid DNA was either applied to two spots on the back, or injected into the gun spots on the abdomen. Panel A: OspC specific IgG1 and IgG2a titers of sera taken from individual mice 2 weeks after the final immunization as determined by ELISA. Data are presented as mean ± S.E.M. of titers for each group ($n = 5$). Panel B: levels of IFN-γ produced by splenocyte cultures upon re-stimulation with 20 mg/ml of OspC or ovalbumin were evaluated by sandwich ELISA of culture supernatants. Results are shown as mean ± S.E.M.

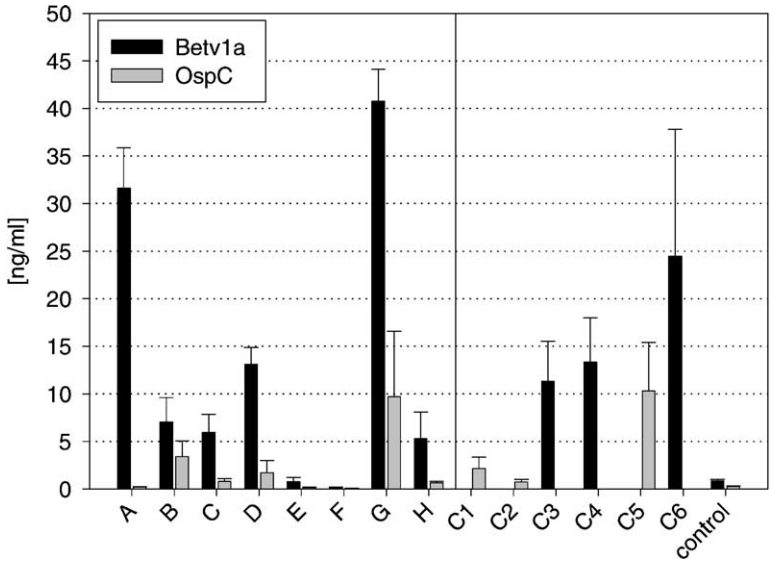

Fig. 4. Antigen-specific induction of IFN-γ in vitro. Levels of IFN-γ produced by splenocyte cultures upon re-stimulation with 20 μg/ml of the respective antigens were evaluated by sandwich ELISA of culture supernatants. Control group consisted of naive mice. Groups Co1–Co6 were only tested for homologous antigen. The results are presented as mean ± S.E.M.of each group ($n = 5$, groups A–H; $n = 3$, groups Co1–Co6; $n = 2$ for control). See Table 1 for description of individual groups.

injection site. Mice immunized i.d. with pCMV-OspC alone or treated with gold beads at a different site had IgG1:IgG2a values of 1.0 and 1.6, respectively. Application of uncoated gold particles to the pCMV-OspC i.d. injection site produced an IgG1:IgG2a ratio of 20.3:1. This difference was due to a significant decrease in OspC-specific IgG2a titers compared to the group receiving no particle bombardment ($P = 0.01$). No significant change was measured with respect to IgG1.

3.3. *Interferon-γ expression is induced by saline needle immunization and inhibited by gene gun immunization*

IFN-γ levels in supernatants of re-stimulated spleen cells were higher for Bet v 1a compared to OspC (Fig. 4). In principle, needle immunization induced significant IFN-γ levels for both antigens, giving the highest values (group G) with a mixture of both DNA constructs applied i.d. into the same spot. In agreement with the Th2-modulating effects, as demonstrated by the IgG1:IgG2a ratios, in most cases immunization with gene gun reduced the IFN-γ levels of the response induced by needle injection. Interestingly, i.d. Bet v 1a injection into the spot of a gene gun shot with OspC vaccine or vice versa nearly completely abolished IFN-γ secretion of re-stimulated spleen cells (groups E and F). Furthermore, bombardment of the i.d. immunization site with uncoated gold particles significantly reduced the IFN-γ levels ($P < 0.02$) induced by the antigen applied with needle injection of plasmid DNA (Fig. 3B). IL-4 values in all groups were below 50 pg/ml with no significant difference to controls (data not shown).

4. Discussion

Multiple factors influence the Th profile of immune responses induced by DNA vaccination, including method and route of immunization and the nature of the encoded antigen [24]. The induction of Th1-type responses after i.d. and i.m. genetic vaccination has been attributed to the presence of immunostimulatory DNA-sequences containing CpG motifs in the plasmid DNA [2,25,26]. Therefore, the different doses of DNA administered by needle injection and gene gun application (50 μg versus 1 μg) were assumed to be responsible for the stimulation of different immune response types [7].

In the present investigation, we studied the influence of the immunization method (i.d. injection and/or gene gun) on the immune responses elicited to a DNA vaccine for an allergen (Bet v 1a) and a spirochetal protein (outer surface protein C, OspC, from *Borrelia burgdorferi* s.l.). Both vaccines contained similar numbers of CpG motifs. The Bet v 1a vaccine had 38 CpG motifs, but none from the *Bet v 1a* gene itself, and the OspC vaccine had 44 CpG motifs, only six of which derived from the *ospC* gene (data not shown). When administered alone, both vaccines elicited the Th-type responses anticipated. Needle immunization (i.d.) triggered a Th1-type response and gene gun immunization a Th2-type response. However, the OspC vaccine produced a relatively weak Th1 response and a strong Th2 response whereas the Bet v 1a vaccine produced a strong Th1 response and a relatively weak Th2 response. This confirms earlier findings that the nature of the antigen may influence the type of immune response made [27].

If the difference of the type of immune responses induced by gene gun (Th2) versus needle injection (Th1) was depen-

dent on the amount of DNA [7,8], then co-injection of large amounts of extraneous DNA by i.d. inoculation should drive the immune response to an independent antigen administered by gene gun away from a Th2 response and towards a Th1 response. The predicted effect was observed when the *ospC* gene was administered by gene gun. In the presence of large amounts (50 μg) of an unrelated DNA (pCMV-Bet), the ratio of IgG1:IgG2a was reduced compared to *ospC* given alone by gene gun. However, in the reciprocal experiment, where pCMV-Bet was given by gene gun there was little or no effect. There does not seem to be a simple relationship between the amount of DNA and the Th-type of the immune response made. The shift to a Th1 response was more marked for the Th2-biased antigen (OspC).

Unexpectedly, the application of extraneous DNA by the gene gun modified the immune response to another antigen given by needle injection. The IgG1:IgG2a ratio was elevated after gene gun co-injection. This switching from a Th1 to a Th2 response was also evident when the needle injection and the gun shot immunizations were done at the same site. The IgG1:IgG2a ratio was elevated after gene gun co-injection for both antigens. These results clearly demonstrate that the immune response to a DNA vaccine administered i.d. can be modulated by a second DNA vaccine given by gene gun.

These findings are further supported by the amount of IFN-γ in supernatants of re-stimulated splenocytes in vitro. Needle immunization promoted higher IFN-γ levels compared to gene gun in all groups except E and F. Here, i.d. and gene gun immunization into the same spot completely abrogated IFN-γ production, indicating the importance of the microenvironment at the injection site.

Our results suggest that gene gun bombardment of the skin may provide for a danger signal [28,29], which even over-rules the strong Th1-promoting signals of immunostimulatory CpG motifs. These observations are indirectly confirmed by recent publications. Co-injection of CpG-oligodeoxynucleotides (CpG-ODN) together with the allergen genes *Bet v 1a* and *Phlp5* did not significantly change the serological profile of a gene gun response. CpG-ODN injection plus gene gun application even increased the IgE titers against Bet v 1a in comparison to gene gun treatment alone [22]. DNA vaccines encoding measles virus hemagglutinin (Th2-biased antigen) and nucleoprotein (Th1-biased antigen) both elicited a dominant Th2-type response after co-immunization via gene gun [30]. The gene gun effect also over-ruled the Th1 promotion of membrane-bound versions of influenza and measles virus hemagglutinins [18].

A possible explanation for the gene gun phenomenon comes from a study, which showed that the gene gun bombardment process itself induces an increase and the migration of activated dendritic cells (DCs) at the respective sites and leads to enlargement of draining lymph nodes [31]. The authors speculate that the physical insult of gold particle bombardment provides for a danger signal that may promote inflammatory cytokine/chemokine reactions. Our data are consistent with a bombardment-inherent danger signal, however, do not support the hypothesis of a Th1-biased inflammatory signal. This can be derived from our experimental design of one antigen being injected i.d. into the back and the second antigen applied via gene gun to the abdomen (groups A and B). Because the antigens are not related and exhibit no cross-reaction, obviously the gene gun shot itself represents a “sort of local adjuvant” for a simultaneously injected antigen. The effect seems to be partly systemic as indicated by the influence of the abdominal gene gun shot on the i.d. needle response induced by injection into the back (compare groups A and E).

A possible explanation for this mainly Th2-type immunomodulating effect of the gene gun could be the stimulation of DC subsets, which preferentially induce Th2-cells. It was recently speculated that different microbial products might induce distinct types of adaptive immune responses by differential activation of murine DC subsets [32,33]. In analogy to this assumption, it is also conceivable that administration of gold particles by gene gun may induce a specific danger signal for the development and activation of Th2-type inducing DC subsets (or suppressing a Th1-inducing signal from DCs).

In summary, the induction of a predominantly Th2-type immune response through genetic immunization with the gene gun is not the consequence of “not enough” Th1-stimulating DNA containing CpG motifs, as hitherto postulated, but the bombardment procedure itself promotes a strong Th2-type response. It is conceivable that this “adjuvant effect” could be responsible for the fact that gene gun inoculation has been proven to be superior to other injection methods in nearly all cases where different methods had been compared [3,34–36]. This has not only been demonstrated for various experimental systems dealing with infectious diseases, gene gun immunization also represents a superior method to induce antigen-specific cytotoxic immune responses in experimental tumor systems with both DNA vaccines encoding entire genes as well as with mini-genes encoding single T-cell epitopes or polytopes (reviewed by Leitner et al. [24]). At present, it is difficult to understand how a Th2-biased method like gene gun application can induce more efficient CTL responses than a Th1-dominated approach like i.d. or i.m. injection. So far, this obvious discrepancy with respect to the Th1/Th2-concept has neither been specifically addressed nor been clarified. Future studies should therefore, further elucidate the mechanisms underlying these observations and evaluate the usefulness and potency of this “novel” danger signal for vaccine development and design.

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