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IgG-Mediated Anaphylaxis to a Synthetic Long Peptide Vaccine Containing a B Cell Epitope Can Be Avoided by Slow-Release Formulation

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Synthetic long peptides (SLP) are a promising vaccine modality to induce therapeutic T cell responses in patients with chronic infections and tumors. We studied different vaccine formulations in mice using SLP derived from carcinoembryonic Ag. We discovered that one of the SLP contains a linear Ab epitope in combination with a CD4 epitope. Repeated vaccination with this carcinoembryonic Ag SLP in mice shows improved T cell responses and simultaneously induced high titers of peptide-specific Abs. These Abs resulted in unexpected anaphylaxis after a third or subsequent vaccinations with the SLP when formulated in saline. Administration of low SLP doses in the slow-release vehicle IFA prevented the anaphylaxis after repeated vaccination. This study underscores both the immunogenicity of SLP vaccination, for inducing T cell as well as B cell responses, and the necessity of safe administration routes. The Journal of Immunology, 2014, 192: 5813–5820.

Adaptive T cell immunity is important in the fight against chronic infections and tumors. Adoptive transfer of tumor-specific CD4 or CD8 T cells can be effective in eradicating tumors in experimental models and in patients (1–5). However, more recently, adoptive transfer of TCR transgenic CD4 or CD8 T cells in combination with vaccination in experimental mouse models has shown severe side effects associated with expansion to very high numbers of tumor-specific T cells (6–8). It could thus be safer to vaccinate to expand endogenous T cells, which are unlikely to proliferate to such dangerously high levels. We therefore vaccinated mice with synthetic long peptides (SLP), which have the capacity to induce CD4 and CD8 T cells dependent on the presence of CD4 and/or CD8 T cell epitopes in a stretch of 25–30 aa.

Vaccination with SLP has shown high immunogenicity in experimental mouse models (9) and in patients with premalignant disease (vulvar intraepithelial neoplasia) induced by human papillomavirus type 16 (HPV16) (10). Repeated SLP vaccination in human beings has shown the induction of Abs after multiple vaccinations (11). We now report unexpected systemic hypersensitivity observed after repeated SLP vaccination in an experimental mouse model using the human carcinoembryonic Ag (CEA) as a model system (12). We studied different formulations of CEA SLP vaccines in water-in-oil slow-release depots (IFA) or in aqueous solutions and the necessity of repeated administration, as performed in the clinical trials. CEA-specific T cell responses were enhanced after repeated SLP vaccination. High levels of peptide-specific IgG Abs were observed after vaccination with a peptide containing a CD4 and Ab epitope. These Abs were associated with hypersensitivity after the third or fourth SLP vaccination of one particular CEA peptide formulated in aqueous solution. Our results emphasize that SLP vaccination is a powerful vaccination approach to generate both T cells and Abs, dependent on the presence of T and B cell epitopes. Slow-release formulation and dose selection are crucial to avoid systemic hypersensitivity.

Materials and Methods

Mice

Female C57BL/6 mice, 6–8 wk old, were purchased from Charles River Laboratories (L’Arbresle, France).Fcγ RI, II, III, and IV knockout (KO) mice were a kind gift of T. Saito, Yokohama (13), Japan, and H. Oettgen, Boston, MA (14), respectively. The FcγRIII KO was generated in our laboratory (15), whereas the FcγRI/III/IV KO mice were recently generated in the Verbeek laboratory with an approach similar to that described by Smith et al. (16): conditional targeting of the FcγRIIb and FcγRII genes, followed by deletion using Cre recombinase of the genomic DNA between the two most distal LoxP sites, spanning the main part of the FcγRIIb and FcγRII genes and the complete FcγRIIb gene, resulting in a FcγRIIb triple KO mouse that was crossed with our FcγRII KO mouse (17). All mice were housed at the animal facility of the Leiden University Medical Center under specific pathogen–free conditions. The animal experiments have been reviewed and approved by the animal ethical committee of Leiden University.

SLP vaccination

Two SLP were chosen on the basis of the presence of either a strong CD4 epitope (SLP-CEA410: VLYGPDDPTI GIQNSVSA NRSDPVTLDV) (18) or both a CD4 and a CD8 epitope (SLP-CEA570: GIQNSVSA NRSDPVTLGDV) (CD8 epitope is delineated in bold letters; CD4 epitope is underlined) (18, 19). SLP were synthesized by our in-house peptide synthesis facility, as described before (20). SLP were dissolved in DMSO. For each vaccination, 7 nmol SLP-CEA410 and 7 nmol SLP-CEA570 were divided in PBS with 20 μg CpG (ODN1826; Invivogen). This mixture was emulsified in IFA (50:50 v/v) and subsequently administered sc. The SLP vaccine was formulated in PBS or IFA, as indicated. Mice were vaccinated two, three, or four times with 2-wk intervals. Directly after the third and fourth vaccination, mice were observed for hypersensitivity.

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Abbreviations used in this article: CEA, carcinoembryonic Ag; HPV, human papillomavirus; KO, knockout; SLP, synthetic long peptide; WT, wild-type.

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Hypersensitivity response

To determine the presence of a hypersensitivity response after vaccination, mice were observed directly after vaccination. Every 5 min, mice were scored for their activity (e.g., eating, interaction, mobility) and general appearance, such as tremors, ruffled fur, and respiratory depression. If mice were inactive and showed signs of ruffled fur, tremors, or respiratory depression, they were considered symptomatic. In selected groups of mice, blood pressure measurements were performed to assess the nature of the hypersensitivity response (see below).

Blood pressure monitoring in mice

The mice were anesthetized, using 4% isoflurane in 17% O₂ and 83% N₂O for induction, which was lowered to 1% isoflurane for maintenance. The depth of anesthesia was monitored carefully throughout the experiment by regular check of the pedal reflex and by constant monitoring of blood pressure. After induction of anesthesia, the femoral artery was catheterized by the following method. An incision was made in the region of the left leg, after which the femoral artery was separated from the vein and proximal/distal ligatures were placed. A metallic clamp was placed in the most proximal region to prevent bleeding. A perpendicular incision was made and the catheter was inserted into the artery. At the end of surgical preparation, blood pressure was allowed to stabilize for ≥5 min. Subsequently, the mice were injected s.c. with 200 μl SLP-CEA410 or SLP-CEA570 (control), which was diluted in saline. Blood pressure was monitored for ≥30 min after SLP-CEA injection, using a physiological pressure transducer (AD Instruments, Colorado Springs, CO). The signal was acquired and digitized in PowerLab (AD Instruments), sampled at 200 Hz, and analyzed offline using LabChart (AD Instruments).

Serum transfer and Ab purification

To determine the contribution of Abs to hypersensitivity, serum transfer was performed. To that purpose, mice were vaccinated twice with SLP-CEA410 or SLP-CEA570, as described above; priming was administered in IFA, and booster vaccination in PBS. At 14 d after the last vaccination, serum was harvested from the mice.

Part of the serum from SLP-CEA410–vaccinated mice was purified using protein-A beads. In short, protein-A beads were washed, resuspended in
the serum, and incubated for 1 h at room temperature. Supernatant was removed, and beads were washed thoroughly with PBS. Igs were separated from the beads by addition of 1 M glycine (pH 2.7). The purified Igs were concentrated, and glycine was exchanged for PBS using a YM30 concentration tube (Amicon). The purified serum contained ∼400 mg Igs per milliliter, as determined by the Bradford assay with BSA as standard. The presence of IgG1 and IgG2b was confirmed by ELISA (data not shown).

A total of 200 ml purified or nonpurified serum was injected i.v. into mice on 2 consecutive days. The next day, mice received a vaccination with either SLP-CEA410 or SLP-CEA570 in PBS, and the mice were scored for their motile activity and general appearance.

**In vitro analysis of T cell responses**

Splenocytes were harvested 10 d after the last vaccination, as described before (12). A total of 4 × 10⁵ splenocytes were incubated with 5 µg/ml SLP-CEA410 or SLP-CEA570. After 2 h, 5 µg/ml brefeldin A (Sigma-Aldrich) was added and cultures were incubated overnight at 37°C/5% CO₂. For detection of cytokines, cells were first fixed and permeabilized using fixation and permeabilization buffer (BioLegend) according to the manufacturer’s protocol. Subsequently, cells were incubated with anti-CD8–FITC, anti-CD4–PerCP, and anti–IFN-γ–allophycocyanin (all from eBioscience). After 30 min incubation on ice, the cells were washed and acquired on a FACSCalibur using Cell Quest (BD). Analysis was performed using FlowJo 7.6.3 (TreeStar).

**Ab detection**

MaxiSorp plates (Nunc) were coated with 2 µg/ml SLP-CEA410, SLP-CEA570, or BSA as a control in coating buffer (sodium carbonate buffer, pH 9.6). Between all incubation steps, plates were washed thoroughly with PBS containing 0.05% Tween 20. After overnight incubation at room temperature, plates were blocked with PBS/1% BSA/0.05% Tween 20 for 1 h at 37°C. Serial dilutions of the serum samples were incubated for 1 h at 37°C. To detect different Ab isotypes, plates were subsequently incubated with anti-IgG1–HRP, anti-IgG2a–HRP, anti-IgG2b–HRP, or anti-IgE–HRP (all from Southern Biotech) for 1 h at 37°C. The presence of Abs was subsequently detected using TMB (Sigma Aldrich), and the color reaction was stopped using 2N H₂SO₄. OD was measured at 450 nm.

**Statistical analysis**

Statistical significance of differences in the treatments of mice was calculated by the Mann–Whitney U test or ANOVA. Results were considered significant at *p < 0.05 and **p < 0.01. Statistical analysis was performed using Prism 5 (GraphPad Software).

**Results**

**Repeated SLP vaccination improves the magnitude of the T cell response**

We studied the effect of repeated s.c. SLP vaccination on the magnitude of the CD4 T cell response in the spleen, as determined by the percentage of IFN-γ–producing CD4 T cells after overnight restimulation with the peptide (Fig. 1). SLP-CEA410 contains a CD4 epitope, whereas SLP-CEA570 contains a CD4 and CD8 epitope (18, 19). Boost vaccinations were formulated either in PBS (Fig. 1B, 1D) or in IFA (Fig. 1C, 1E). T cell responses were not detected after the first vaccination in IFA (data not shown). CD4 T cell responses against SLP-CEA410 were much higher than the CD4 responses raised against SLP-CEA570. The response against the latter did not increase after even more repetitions of SLP vaccination. When all vaccinations were formulated in IFA, an increase in the CD4 T cell response was observed after each boost. However, the magnitude of the response in blood was lower compared with boost vaccinations in PBS. In contrast, CD4 responses against SLP-CEA570 were undetectable after administration in PBS. CD8...
T cell responses against SLP-CEA570 were detectable after vaccinations in either IFA or PBS (data not shown).

Repeated immunization of mice with SLP-CEA410 formulated in PBS can induce hypersensitivity

Despite our experience that vaccination with many different SLP sequences showed no obvious side effects, we observed that mice given a third or fourth vaccination in PBS with SLP-CEA410 showed signs of hypersensitivity within 30 min after vaccination. The symptoms included reduced mobility, respiratory depression, tremors, and ruffled fur. If the vaccine was formulated in IFA, no symptoms were observed. In the majority of cases, the mice recovered without any remaining symptoms. Histological examination of the organs of symptomatic mice did not show any signs of organ disease (data not shown). In Fig. 2, the percentages of mice symptomatic after the third or fourth vaccination formulated in PBS or IFA are represented. Whereas almost 90% of the mice were symptomatic if SLP-CEA410 was formulated in PBS, <10% were symptomatic if SLP-CEA410 was formulated in IFA \((p = 0.006,\) Mann–Whitney \(U\) test). In contrast, mice vaccinated with SLP-CEA570 did not show signs of hypersensitivity at all if vaccinated with PBS or IFA.

Although T cell numbers were not exceedingly high after two vaccinations, we analyzed blood from symptomatic mice to determine the presence of IFN-\(\gamma\) and TNF-\(\alpha\). No elevated levels of IFN-\(\gamma\) or TNF-\(\alpha\) were present, excluding cytotoxicity mediated as observed by Kitamura et al. (6) and Ly et al. (7). Blood samples drawn at 0 and 6 h post vaccination were analyzed in a Luminex assay to determine whether mice suffered from a cytokine storm. All cytokine levels were within the background cytokine levels of naive mice (data not shown), which excluded a cytokine storm as being responsible for the observed side effects.

Because SLP vaccination in nonhuman primates and patients has shown the induction of peptide-specific Abs in some cases (21), we analyzed the presence of Abs against the respective CEA SLP. To that purpose, an ELISA was performed in which plates were coated with SLP-CEA410 and SLP-CEA570 and subsequently incubated with serum obtained from vaccinated mice. With the use of HRP-labeled anti-IgG subclass and anti-IgE–specific Abs, IgG1, IgG2a, and IgG2b, but not IgE, against SLP-CEA410 were detected in mice vaccinated with this SLP (Fig. 3). Priming in PBS did not induce high titers of peptide-specific Abs (data not shown). In contrast, when priming was formulated in IFA, high titers of peptide-specific Abs were induced, regardless of whether the boost was formulated in IFA or PBS (Figs. 3, 4). SLP-CEA410 thus contains an Ab epitope in addition to the CD4 epitope. In conclusion, dependent on the formulation of the vaccine, high titers of peptide-specific Abs can be induced with a SLP containing a B and T cell epitope.

Abs are responsible for hypersensitivity after repeated SLP vaccination

The presence of high levels of IgG suggests a role for these Abs in the acute side effects after repeated vaccination with SLP-CEA410. Therefore, we performed a serum transfer experiment. Naive mice received serum from either SLP-CEA410– or SLP-CEA570–vaccinated mice and were subsequently vaccinated with 35 nmol of either SLP-CEA410 or SLP-CEA570 and monitored for symptoms of hypersensitivity (reduced mobility, respiratory depression, tremors, and ruffled fur) (Fig. 5). Because Ab titers in these mice were expected to be lower, we administered a higher dose of SLP for the challenge. Mice that received ProtA purified or nonpurified serum from SLP-CEA410–vaccinated mice (SLP-CEA410 serum) before subsequent SLP-CEA410 vaccination were all symptomatic within 15 min after vaccination. In contrast, mice that were injected with serum from SLP-CEA410–vaccinated mice and received an SLP-CEA570 vaccination were completely healthy, as were mice that received serum from SLP-CEA570–vaccinated mice (SLP-CEA570 serum) and a subsequent vaccination with either SLP-CEA410 or SLP-CEA570. These results clearly show that Abs are involved in the side effects observed after repeated SLP-CEA410 boosting in PBS.

Administration of low-dose SLP in IFA prevents hypersensitivity

As shown in Fig. 2, the formulation of 7 nmol SLP-CEA410 in IFA did not result in side effects. This might be due to slow release of the SLP from the s.c. depot and thereby reduce the actual amount of diffusing free SLP that might bind to the circulating Abs. To study whether the side effects are related to the injected dose, we
increased the injected SLP dose formulated in IFA $\leq 105$ nmol. Mice were primed with 7 nmol SLP-CEA410 in IFA and boosted with 7 nmol SLP-CEA410 in PBS. Mice were subsequently challenged with 7 nmol SLP-CEA410 in PBS or 18, 36, 72, or 105 nmol SLP-CEA410 in IFA and monitored for signs of hypersensitivity. Results are shown in Fig. 6. At the highest dose formulated in IFA, all mice were symptomatic within 20 min after the last vaccination. With decreasing concentration, a reduction in the percentage of symptomatic mice was observed. The gray bar represents the mice that received 7 nmol SLP-CEA410 in PBS. Upon formulation in IFA, a 10 times higher dose of SLP can be administered to observe a comparable percentage of symptomatic mice. Therefore, slow-release formulation in IFA prevents major side effects at lower SLP doses, which were still able to effectively immunize for the induction of T cell responses (not shown).

Role of FcR in Ab-mediated toxicity

We have shown that Abs are involved in the hypersensitivity observed after repeated SLP vaccination in PBS. Systemic anaphylactic shock is associated with drastic hypotension (22); therefore, we analyzed blood pressure in vaccinated mice displaying hypersensitivity compared with control vaccinated nonsymptomatic mice. (Fig. 7A) The drop in blood pressure after injection with 7 nmol SLP-CEA410 was 60% compared with control mice, and mice. (Fig. 7A) The drop in blood pressure after injection with lactic shock is associated with drastic hypotension (22); therefore, served after repeated SLP vaccination in PBS. Systemic anaphy-

We have shown that Abs are involved in the hypersensitivity observed after repeated SLP vaccination. Serum was harvested from mice that received two vaccinations with either SLP-CEA410 or SLP-CEA570. The serum was subsequently transferred to naive mice receiving 200 µl serum i.v. from mice vaccinated with SLP-CEA410 (closed symbols) or SLP-CEA570 (open symbols) in PBS. Abs were purified using protein A beads from serum of SLP-CEA410–vaccinated mice. Mice were observed after vaccination for the presence of side effects. Percentage of mice expressing side effects is indicated. Symptoms include reduced mobility, respiratory depression, tremor, and ruffled fur. Representative experiment of two independent experiments, $n = 3$ mice per group.

FIGURE 5. Abs are involved in side effects observed after repeated SLP vaccination. Serum was harvested from mice that received two vaccinations with either SLP-CEA410 or SLP-CEA570. The serum was subsequently transferred to naive mice receiving 200 µl serum i.v. from mice vaccinated with SLP-CEA410 (closed symbols) or SLP-CEA570 (open symbols) in PBS. Abs were purified using protein A beads from serum of SLP-CEA410–vaccinated mice. Mice were observed after vaccination for the presence of side effects. Percentage of mice expressing side effects is indicated. Symptoms include reduced mobility, respiratory depression, tremor, and ruffled fur. Representative experiment of two independent experiments, $n = 3$ mice per group.

Discussion

In this study, we show that repeated vaccination with an SLP containing both a CD4 and a B cell epitope can induce SLP-specific IgG Abs that can mediate SLP-induced hypersensitivity. Ab titers increased after each vaccination (data not shown) regardless whether the booster injections were formulated in PBS or IFA. Serious hypersensitivity occurred only after the third or fourth subsequent injection formulated in PBS, suggesting rapid Ab–SLP immune complex formation, which was causal in inducing the observed side effects. The original aim of our study was the analysis of the necessity of repeated vaccination with CEA SLP for the induction of CD4 and CD8 T cell responses. Repeat vaccination resulted in increased T cell responses, although when formulated in PBS, the optimal number seems to be three vaccinations. In contrast, formulation in IFA boosted the response also after four vaccinations. On the basis of our observations with the SLP-CEA410, although in our experience a rare case, we suggest careful attention to dose and formulation of SLP vaccines, especially when administered in PBS, to avoid undesirable hypersensitivity symptoms. The rarity of SLP sequences causing this particular side effect is illustrated by the fact that of 35 SLP sequences tested in our laboratory over the years for induction of CD4 and/or CD8 T cell responses in C57BL/6 mice, only repeated vaccination with SLP-CEA410 has shown this adverse reaction when formulated in PBS. Two other SLP sequences not related to absence of FcγRIII and IgE, and FcγRIII/IgE quadruple KO mice that lack all IgG FcR. FcγRIII/IgE KO mice still displayed hypersensitivity after challenge, albeit milder. In contrast, FcγRI/II/III/IV KO mice showed no signs of hypersensitivity (Fig. 7C). This was not caused by differences in Ab levels between FcγRI/II/III/IV KO mice and C57BL/6 mice. Serum analysis revealed similar levels of total IgG in both strains, with titers in the KO mice even slightly higher than in the WT mice (Fig. 7D). When analyzed for the different isotypes, levels are comparable between FcγRI/II/III/IV KO mice and C57BL/6 KO mice, except for IgG2a levels, which are slightly lower in the KO mice (Supplemental Fig. 1).

By combining these observations, it can be concluded that IgG-mediated, but not IgE–mediated, hypersensitivity can play a role after repeated SLP vaccination and that FcγR play a crucial role in these side effects. Because the IgG1 titers are high in vaccinated mice, a dominant role for FcγRII in the observed hypersensitivity could be expected. However, FcγRIII/IgE double KO were still affected. This finding indicates that there is no exclusive role for FcγRII, suggesting that other FcγR on other cell types than mast cells or basophils are also involved.

To further explore the role of FcγR, we analyzed FcγRIII/IgE KO mice that lack possible activation of mast cells or basophils owing to absence of FcγRIII and IgE, and FcγRII/III/IV quadruple KO mice that lack all IgG FcR. FcγRIII/IgE KO mice still displayed hypersensitivity after challenge, albeit milder. In contrast, FcγRI/II/III/IV KO mice showed no signs of hypersensitivity (Fig. 7C). This was not caused by differences in Ab levels between FcγRI/II/III/IV KO mice and C57BL/6 mice. Serum analysis revealed similar levels of total IgG in both strains, with titers in the KO mice even slightly higher than in the WT mice (Fig. 7D). When analyzed for the different isotypes, levels are comparable between FcγRI/II/III/IV KO mice and C57BL/6 KO mice, except for IgG2a levels, which are slightly lower in the KO mice (Supplemental Fig. 1).

By combining these observations, it can be concluded that IgG-mediated, but not IgE–mediated, hypersensitivity can play a role after repeated SLP vaccination and that FcγR play a crucial role in these side effects. Because the IgG1 titers are high in vaccinated mice, a dominant role for FcγRII in the observed hypersensitivity could be expected. However, FcγRIII/IgE double KO were still affected. This finding indicates that there is no exclusive role for FcγRII, suggesting that other FcγR on other cell types than mast cells or basophils are also involved.

FIGURE 5. Abs are involved in side effects observed after repeated SLP vaccination. Serum was harvested from mice that received two vaccinations with either SLP-CEA410 or SLP-CEA570. The serum was subsequently transferred to naive mice receiving 200 µl serum i.v. from mice vaccinated with SLP-CEA410 (closed symbols) or SLP-CEA570 (open symbols) in PBS. Abs were purified using protein A beads from serum of SLP-CEA410–vaccinated mice. Mice were observed after vaccination for the presence of side effects. Percentage of mice expressing side effects is indicated. Symptoms include reduced mobility, respiratory depression, tremor, and ruffled fur. Representative experiment of two independent experiments, $n = 3$ mice per group.
CEA (murine leukemia virus and HIV sequences) showed side effects when repeatedly administered in PBS.

Although CD8 T cells are the main cytotoxic T cells that can exert an effect on tumor or virus-infected cells, CD4 T cells are important in providing help to the CD8 T cells during priming and effector phases of the immune response (24, 25). Furthermore, CD4 T cells themselves can also contribute to antitumor activity (26–29), and in our recent clinical vaccination trial with SLP of HPV oncoproteins, the strength of the CD4 T cell response as determined ex vivo correlated positively with the clinical response in patients carrying premalignant lesions caused by high-risk HPV (10, 30). Long peptides have an advantage over short or minimal binding epitope peptide in that they need to be internalized and processed by professional APCs (31). In the current study, the presence of a potent CD4 T cell epitope also provided help for the generation of peptide-specific B cells and the production of peptide-specific IgG Abs owing to the simultaneous presence in the SLP of a potent B cell epitope.

The classical pathway of anaphylaxis is mediated by mast cells, IgE, and histamine (22, 32). We were unable to detect IgE in the serum of vaccinated mice, and our experiments with FcR KO mice implicated IgG, but not IgE, as being involved in causing the observed hypersensitivity. Several papers have described IgG-mediated anaphylaxis in mice (23, 32–34).

Our study emphasizes that caution is needed when using SLP containing linear immunogenic B cell epitopes for vaccination. The presence of a B cell epitope is not unique for the CEA-SLP410, Because we also observed SLP-specific IgG Abs after repeated vaccination with two other SLP that showed hypersensitivity. These peptides were also derived from exogenous Ags and contained CD4 epitopes for C57BL/6 (H-2b) mice. Furthermore, Abs have also been detected in patients vaccinated with p53-SLP.

### FIGURE 6
Administration in IFA prevents hypersensitivity at low dose. Mice were primed with 7 nmol SLP-CEA410 with 10 μg CpG in IFA. First boost was formulated in PBS. Subsequently, mice were vaccinated with titrating concentrations of SLP-CEA410 in IFA (black bars). As a control, mice were vaccinated with 7 nmol SLP-CEA110 PBS (gray bar). Mice were observed for side effects after the third vaccination. Percentage of mice showing sign of hypersensitivity at t = 30 min after vaccination is indicated. All mice symptomatic at t = 30 min remained so for at ≥30 min. Symptoms include reduced mobility, respiratory depression, tremor, and ruffled fur. n = 4 mice per group.

### FIGURE 7
The role of FcR in side effects. Mice were primed with 7 nmol SLP-CEA110 in IFA and subsequently boosted twice with 7 nmol in PBS. To induce side effects after the fourth vaccination, 35 nmol SLP-CEA410 in PBS was used. (A) Blood pressure in mice after vaccination. Three mice per group were vaccinated with SLP-CEA410 or SLP-CEA390, and blood pressure was measured for 30 min after vaccination. (B) CD16/IgE KO mice (lack Fc γRIII and IgE [gray triangles]) and FcRg chain KO mice (lack all stimulatory FcR [open squares]). (C) FcgRI/II/III/IV KO mice (lack all Fcg receptors [gray diamonds]) and FcRg chain KO mice (lack all stimulatory FcR [open squares]). WT C57BL/6 mice were vaccinated (black circles) as control. Mice were observed and scored for signs of hypersensitivity directly after the fourth vaccination. Percentage of mice that were symptomatic is indicated. Symptoms include reduced mobility, respiratory depression, tremor, and ruffled fur. (D) Total IgG levels in serum of Bl/6 compared with Fc FcgRI/II/III/IV KO mice. n = 10 mice per group, experiments representative of two independent experiments.
(11) or HPV-SLP and nonhuman primates (21) (P. Mooij, J.W. Drijfhout, S. van der Burg, C. Melief, and G. Koopman, personal communication). The presence of these SLP-specific Abs in humans and nonhuman primates did not correlate with hypersensitivity after repeated vaccination, possibly because these vaccinations used formulation of peptides in mineral oil Montanide ISA 51.

Recently, Hailemichael et al. (35) described slow-release formulations like Montanide ISA-51 and IFA as acting like T cell sinks in tumor-vaccination settings. However, this effect occurred only when short, minimal epitopes were used, whereas with long peptides, like the ones we use in our experiments, this phenomenon was absent and good antitumor responses were induced. Combined with our findings that slow-release formulations can decrease the changes of IgG-mediated side effects, we conclude that for SLP vaccinations, IFA/Montanide ISA-51 formulations are not only suitable but also preferred to saline.

It is difficult to extrapolate our observations to human beings or nonhuman primates because of the very large difference in effectively immunizing peptide dose relative to the size of the organism. For instance, we have used 20 or 50 μG SLP for each effective vaccination, whereas in human patients and nonhuman primates 300 μg per individual SLP is used (10, 36). In contrast, comparable amounts of Ag–Ab complexes in humans might cause less severe toxicity, probably owing to larger body size. Indeed, in recent clinical studies with SLP only mild local toxicities were observed (10), some of which may have been Ab mediated. Furthermore, differences in the Ab subtype indication could also influence the clinical outcome. Therefore, we cannot directly extrapolate our data to human vaccination, but our current results show that careful attention has to be given to potential systemic toxicity mediated by Abs after SLP vaccination, which warrants optimizing dose and mode of administration. In particular, formulation in a slow-release system prevents these side effects at immunizing doses, as already demonstrated in human and nonhuman primate studies (10, 36).

Disclosures

The authors have no financial conflicts of interest.

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7. Hailemichael, F., M. R. Offringa, S. H. van der Burg, and M. J. Welters. 2001. Combined with our findings that slow-release formulations can decrease the changes of IgG-mediated side effects, we conclude that for SLP vaccinations, IFA/Montanide ISA-51 formulations are not only suitable but also preferred to saline.


Supplemental Figure 1

Legend: Serum titers of knock out mice. Mice were vaccinated three times with 7 nmol SLP-CEA410 with 10 ug CpG. Priming was formulated in IFA, subsequent boosting in PBS. Serum was taken two weeks after second boost. The presence of peptide-specific antibodies was determined by ELISA as described in the Materials and Methods. Total IgG of Fc gamma chain knock out and CD16/IgE knock out mice (A) IgG1, IgG2b and IgG2a titers in FcR I/II/III/IV knock out mice (B, C, D) Average of 10 mice per group is shown, data from two experiments combined, total of three experiments performed.