

Vaccination with a Modified Vaccinia Virus

Ankara-based vaccine protects mice from allergic sensitization

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Abstract

Background Currently, no treatment is available for food allergy and strict avoidance of the allergenic food remains the only way to manage the allergy. New strategies leading to a safe and efficacious food allergy treatment are required. Modified vaccinia virus Ankara (MVA), which allows high levels of expression of recombinant protein *in vivo* and gives rise to a Th1-biased specific immune response, was used as a prophylactic vaccine in a murine model of ovalbumin (OVA) allergy.

Methods An MVA-OVA vector vaccine was prepared. Female BALB/c mice were vaccinated twice with a MVA-OVA vector vaccine, followed by sensitization with OVA plus alum. OVA-specific immunoglobulin E (IgE) activity was measured by mediator release from rat basophilic leukaemia cells, whereas specific IgG subclass titers were determined by enzyme-linked immunosorbent assay.

Results Expression of immunological active OVA in mammalian cells was demonstrated. OVA-specific IgE levels in sera from MVA-OVA-vaccinated mice were reduced and appeared delayed. The vaccine-mediated immune modulation was dose-dependent; the highest vaccine dose protected 50% of the animals from allergic sensitization. Upon sensitization, similar OVA-specific IgG1 titers were found in all mice, but the OVA-specific IgG2a antibody levels were strongly increased in MVA-OVA-vaccinated mice, signifying a Th1-biased and, non-allergic immune response.

Conclusions Prophylactic vaccination with MVA-OVA delays and in part even prevents the onset of a successful allergen-specific sensitization. Recombinant MVA, which fulfills the requirements for clinical application, is a promising candidate vector for the development of novel approaches to allergen-specific prophylactic vaccination and specific immunotherapy. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords IgE; modified vaccinia virus Ankara; specific immunotherapy

Introduction

The prevalence of food allergy as a life-threatening hypersensitivity reaction appears to have increased in recent years. Approximately 6% of young children and 3% of adults in the USA are believed to suffer from immunoglobulin E (IgE)-mediated food allergy [1,2]. There is clear evidence that food allergies

Received: 26 May 2008

Revised: 15 August 2008

Accepted: 15 August 2008

must be considered as highly relevant health issues in European populations as well. Yet, meta-analysis of more than 900 studies suggest highly varying levels of prevalence (3–35%) [3]. In response to this lack of comprehensive data and comparable study design, an ongoing multicentre study aims at elucidating the prevalence of food allergy across Europe [4].

Classical specific immunotherapy (SIT), which is used successfully to treat allergies to aeroallergens and hymenoptera venoms, is not recommended for treatment of food allergy due to a high risk of severe adverse reactions. Even though a decrease in skin prick test reactivity in controlled trials of SIT for peanut allergy was observed, severe systemic reactions showed an unfavourable risk-to-benefit ratio [5,6]. Although novel therapies for food allergy are intensively investigated [7], the only way to prevent reactions remains strict avoidance of the allergenic food. Because the development of allergic diseases is not only dependent on environmental, but also inheritable factors, prevention of allergy either unspecifically (e.g. probiotics) or specifically (SIT) for children from highly atopic families is an important research issue [8–11].

Even though the exact mechanism underlying successful SIT has not been fully elucidated, the efficacy is thought to depend on the modulation of the existing (Th2-biased) immune response to the allergen into a non-allergic, tolerance-like immune response. This may be mediated by anergy, depletion or regulation of the effector T cells resulting in a healthy immune response, which is defined by a fine balance between the different allergen-specific T cell subtypes [12]. Successful SIT appears to be accompanied by a reduction of T cell and eosinophil recruitment in response to allergen. A shift in the balance of Th1 and Th2 cytokine expression is also observed [12]. These effects may be enhanced by combining an allergen-specific approach with a substance of high immunomodulatory capacity and anaphylactic side-effects may be circumvented by intracellular expression of the allergen.

Poxviruses engineered to express foreign genes are among the most forefront tools for vaccine development [13], whereas the use of recombinant DNA/gene transfer vaccines for prophylaxis or treatment of allergic diseases is just starting to be explored. The modified vaccinia virus Ankara (MVA) was obtained by serial passage in chicken embryo fibroblasts, resulting in the loss of a substantial portion of the genetic information normally encoded by vaccinia viruses. Among the viral gene functions affected in MVA, many are likely involved in the regulation of virus–host interactions [14]. Consequently, MVA has lost its capability to productively infect most mammalian cells [15]. The virus has been found to be safe when clinically tested for immunization against smallpox or evaluated in severely immune-suppressed animals [16–18]. MVA is currently being investigated as a highly promising vector vaccine candidate in preclinical and clinical studies, including vaccines against malaria, HIV, SARS, measles, influenza and cancer [13,19–24].

Similar to all vaccinia viruses, MVA can infect a broad variety of all cell types [25] activating both branches (humoral and cellular) of the immune system. *In vivo*, MVA appears to preferentially enter dendritic cells among hematolymphocytes with evidence for direct antigen presentation [26]. In addition, infection of dendritic cells appears to be linked to the strong *in vivo* induction of type I interferons, a hallmark of the MVA immunostimulatory capacity [27]. Characterization of immune responses induced by recombinant MVA vaccines suggests a clear bias towards a Th1 type and stronger cellular responses directed against the recombinant protein than observed with standard replication competent vaccinia virus [28].

The aim of the present first proof-of-concept study was to determine whether *in vivo* expression of food allergens by recombinant MVA (rMVA) may serve as a novel approach for prophylactic allergen-specific immunotherapy. The results will serve as a basis for future studies of immunotherapy in food allergy models. For this purpose, rMVA expressing ovalbumin (OVA; Gal d 2) from domestic chicken *Gallus domesticus*, a frequently used model allergen, was generated and applied as a prophylactic vaccine in a murine model of OVA allergy to demonstrate its capability for modulation of the OVA-specific humoral immune response.

Materials and methods

Generation of recombinant vector vaccine MVA-OVA

Recombinant MVA-OVA was constructed using previously described vector plasmids and methodologies [29]. Briefly, the gene sequence encoding for chicken OVA was inserted under the transcriptional control of the vaccinia virus early late promoter P7.5 into plasmid pIII_dHR-P7.5. Recombinant viruses were formed in chicken embryo fibroblasts infected with wild-type MVA (wtMVA, from the 582nd passage, cloned isolate F6) and transfected with pIII_dHR-P7.5-OVA DNA. MVA-OVA was plaque isolated on rabbit RK-13 cells (ATCC CCL-37) using transient host range K1L-selection [30] and expression was ascertained by SDS-PAGE and western blot analysis of cytoplasmic extracts from infected BHK cells using polyclonal rabbit anti-OVA antiserum (Acris Antibodies GmbH, Hiddenhausen, Germany; Figure 1B). For preparation of experimental vaccines, MVA-OVA and wtMVA were amplified on hamster BHK-21 cells (ATCC CCL-10), purified by ultracentrifugation through sucrose, titrated and stored as concentrated virus material in 1 mM Tris-HCl, pH 9.0 at –80 °C.

Mice and immunization

Female BALB/c mice (four animals per group; 6–8 weeks of age; Harlan-Winkelmann, Borcheln, Germany) were vaccinated intraperitoneally (i.p.) at day 1 (V1) and day

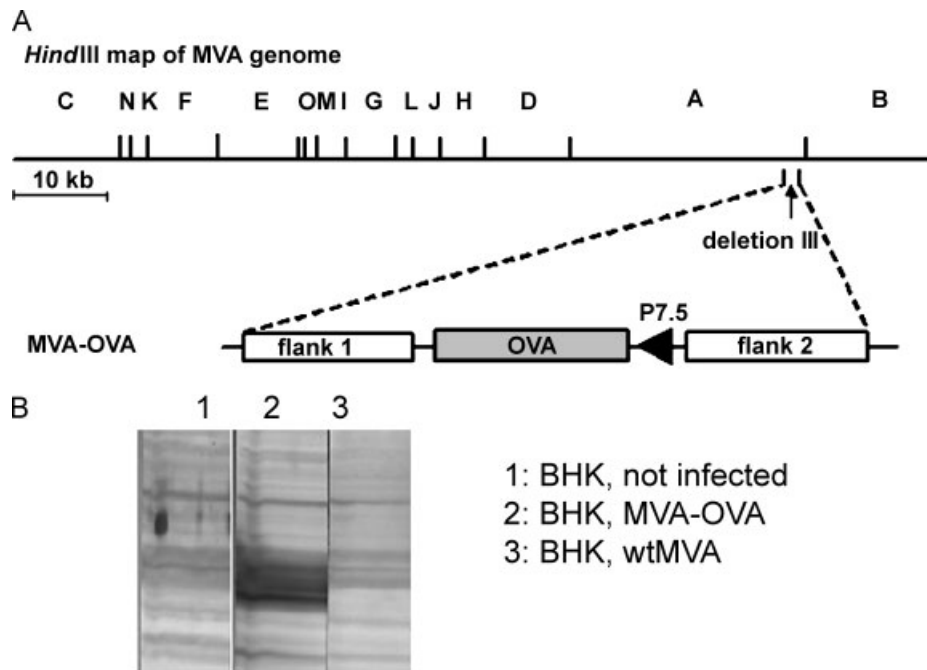


Figure 1. Recombinant gene expression by MVA-OVA. (A) The MVA-OVA vector virus contains the gene sequence encoding for chicken albumin (OVA) under the transcriptional control of the vaccinia virus-specific early/late promoter P7.5. As indicated relative to the *Hind*III restriction map of the MVA genome, this expression cassette has been stably inserted into the site of major deletion III using MVA flanking gene sequences (flank1, flank2) for targeted homologous recombination. (B) Production of recombinant OVA protein upon MVA-OVA infection was ascertained by western blot analysis of: (1) not infected; (2) MVA-OVA infected and (3) wtMVA infected BHK cells using polyclonal rabbit anti-OVA antiserum

28 (V2) with 10^6 or 10^8 infectious units (IU) of MVA-OVA (10^6 IU MVA-OVA, 10^8 IU MVA-OVA) or 10^8 IU wtMVA (10^8 IU wtMVA) diluted in vaccination buffer (10 mM Tris/HCl, 140 mM NaCl, pH 7.4); unvaccinated mice received vaccination buffer only. Intraperitoneal immunization of each vaccination group with doses of 0.1 μ g, 1 μ g, 10 μ g of OVA (Imject OVA, Pierce, Rockford, IL, USA) dissolved in phosphate-buffered saline (PBS) plus 1 mg of alum [$\text{Al}(\text{OH})_3$; Merck, Darmstadt, Germany] starting at week 14 was performed five times every 2 weeks (S1–S5). Blood was drawn from the retro-orbital plexus every 2 weeks. Two weeks after the last immunization, mice were sacrificed and blood was drawn by heart puncture. Sera were stored at -20°C until use. In all experiments animals were handled in compliance with German regulations for animal welfare.

Enzyme-linked immunosorbent assay (ELISA) for the determination of OVA-specific IgG subclass titers

OVA-specific IgG subclass titers were measured by ELISA. Maxisorp plates (Nunc, Wiesbaden, Germany) were coated with 100 μ g/ml OVA (Sigma-Aldrich, Munich, Germany) or dilution series of purified mouse IgG1, IgG2a, IgG2b and IgG3 (BD Pharmingen, Heidelberg, Germany) as internal performance control dissolved in 50 mM sodium carbonate buffer, pH 9.6, respectively. Biotinylated antibodies specific for mouse IgG1 (A85–1), IgG2a (R19–15), IgG2b (R12–3) and IgG3

(R40–82) (BD Pharmingen) were used with streptavidin-peroxidase (Calbiochem, Darmstadt, Germany) and 3,3',5,5'-tetramethylbenzidine (Roth, Karlsruhe, Germany) as substrate. Antibody titers were defined as the reciprocal of the maximum dilution eliciting an optical density (OD) value of background plus three standard deviations. A pool of mouse sera with a high titer of OVA-specific IgG antibodies of all four subclasses served as positive control and reference and was used on each plate.

Beta-hexosaminidase release from rat basophil leukemia cells (RBL)

As functional read-out for the measurement of OVA-specific IgE in sera the beta-hexosaminidase release assay, which is an *in vitro* model of the murine type I reaction, was performed by using the rat basophil leukemia cell line RBL-2H3 [31,32]. Briefly, RBL-2H3 cells were sensitized with 1:75 diluted mouse serum (binding of IgE to $\text{Fc}\epsilon$ receptors on cell surface) and stimulated with seven dilutions of OVA (crosslinking of $\text{Fc}\epsilon$ receptors by binding of antigen to specific IgE) with concentrations ranging from 0.1 ng/ml to 100 μ g/ml leading to mediator release. The release values were calculated as percentage of an internal positive control to compensate for day-to-day variations, which resulted in release values exceeding 100%. The OVA-specific IgE antibody response was characterized by determination of the maximum IgE-mediated release value (reflecting the biological activity of

specific IgE) and the lowest test concentration (0.1 ng/ml to 100 µg/ml of OVA) able to induce mediator release (reflecting the degree of sensitization of the animals; designated as: minimum OVA concentration required for mediator release induction).

Results

Preparation of MVA-OVA and high level intracellular expression of ovalbumin

The recombinant vector vaccine MVA-OVA (Figure 1A) was successfully prepared to high titers. When BHK cells were infected with MVA-OVA and tested with an anti-OVA antibody by western blotting, strong intracellular expression of OVA was observed, whereas no OVA was detectable in cells infected with wtMVA (Figure 1B).

A wide dosage range of OVA resulted in strong specific IgE responses

Sensitization with allergen in mice by i.p. immunization plus the IgE-inducing adjuvant alum depends on the genetic background of the mouse strain, on the antigen, and the dosage used [33,34]. Because BALB/c mouse models of ovalbumin allergy are well characterized and accepted, only three different doses of OVA were used in our model to determine the antigen dose that leads to the most efficient sensitization.

Groups of four unvaccinated mice were sensitized five times in 2-week intervals with 0.1 µg, 1 µg or 10 µg OVA and alum. The control mice received PBS and alum. One week after each immunization, blood was drawn from the retro-orbital plexus.

The RBL assay, an *in vitro* model of the type I allergic reaction, was used to determine particularly those OVA specific IgE antibodies in sera that crosslink Fcε-receptors upon stimulation with the antigen and induce mediator release. Analysis of pooled sera in RBL assay showed that the application of all three doses ranging from 0.1 µg of OVA to 10 µg of OVA per dose elicited a clear OVA-specific IgE antibody response (Figure 2). The sera of mice which received 0.1 µg of OVA were analyzed individually because the lowest dose led to the highest IgE response.

Absence of OVA-specific IgE correlated well with the IgG2a/IgG1 ratio induced by MVA-OVA vaccination

To investigate the effect of a MVA-OVA vaccination, at first individual sera of vaccinated and unvaccinated mice were compared at the time point with maximum IgE response in our dose finding experiment (after S3; Figure 2).

The vaccination with 10⁶ and 10⁸ IU MVA-OVA led to a clear reduction in OVA-specific IgE response compared to

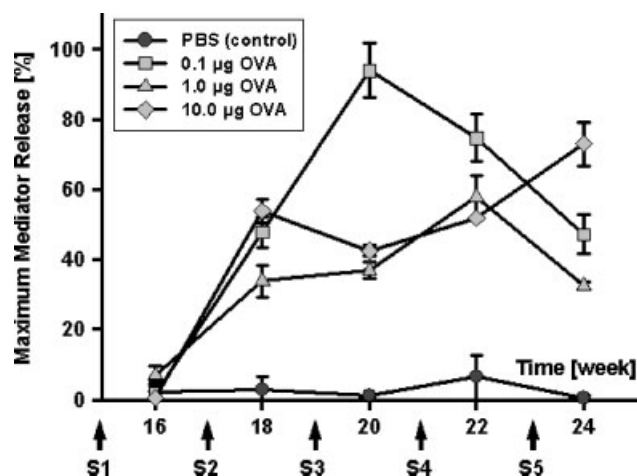


Figure 2. Comparison of IgE antibody responses after sensitization with different OVA doses. OVA-specific IgE reactivity of unvaccinated mice was determined by RBL assay after each sensitization (indicated with arrows, x-axis). Mice were sensitized with 0.1 µg (grey squares), 1 µg (grey triangles) or 10 µg (grey diamonds) of OVA. Control mice received PBS (black circles). Data were obtained from pooled sera of each group and shown with standard deviations of duplicate determinations

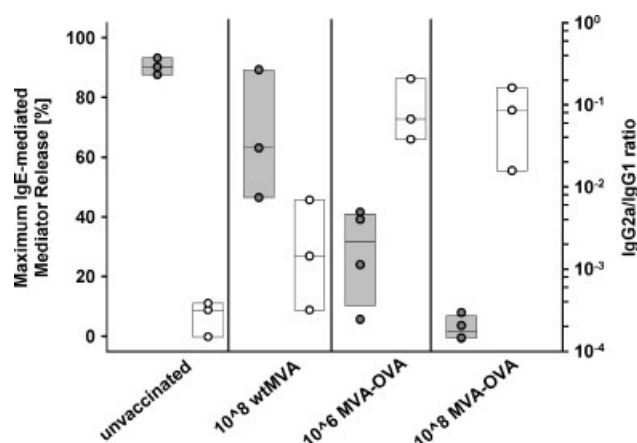


Figure 3. IgE and IgG subclass response after the third sensitization. OVA-specific IgE in individual sera of vaccinated mice was measured by RBL-assay (grey bars), whereas OVA-specific IgG1, IgG2a was measured by ELISA (white bars) and is shown as ratio between IgG2a and IgG1 titers. The bars comprise the value range for each group ($n = 3-4$); black lines indicate the median value

unvaccinated mice. This protective effect depended on the dosage of the vaccine. OVA-specific IgE was not detectable in sera from mice vaccinated with the high dosage (10⁸) until after the third sensitization (S3). Mice vaccinated with low dosage MVA-OVA (10⁶) produced small amounts of OVA-specific IgE and median of the maximum mediator release was approximately 30% (Figure 3, grey bars). In the unvaccinated group high IgE responses were measured, signifying an unimpaired allergic sensitization. Interestingly, two out of three control animals, which received 10⁸ IU wtMVA, showed a decreased OVA-specific IgE response compared to unvaccinated mice, indicating that administration of wtMVA alone may affect

sensitization. Analysis of serum pools from the four other vaccination groups (four animals per group) sensitized with 1 μ g and 10 μ g of OVA revealed an identical dose-dependent delay of sensitization (data not shown).

A high IgG2a/IgG1 ratio, indicating a Th1-biased immune response [35,36], was found in vaccinated mice that showed no or little antigen-specific IgE (Figure 3, white bars). As expected, the IgG2a/IgG1 ratios in control mice that were either unvaccinated or 'vaccinated' with 10^8 IU wtMVA were much lower signifying a more Th2-biased immune response.

MVA-OVA vaccination affects kinetics of OVA-specific IgE response

The IgE response in individual sera was analysed regarding two independent parameters: (i) The maximal mediator release (Figure 4A to 4C) reflects the biological activity of specific IgE, because the intrinsic factors that regulate the magnitude of mediator release are probably identical within a cell line. (ii) The lowest test concentration of OVA able to induce mediator release (Figure 4D to 4F) is a marker for the basophilic

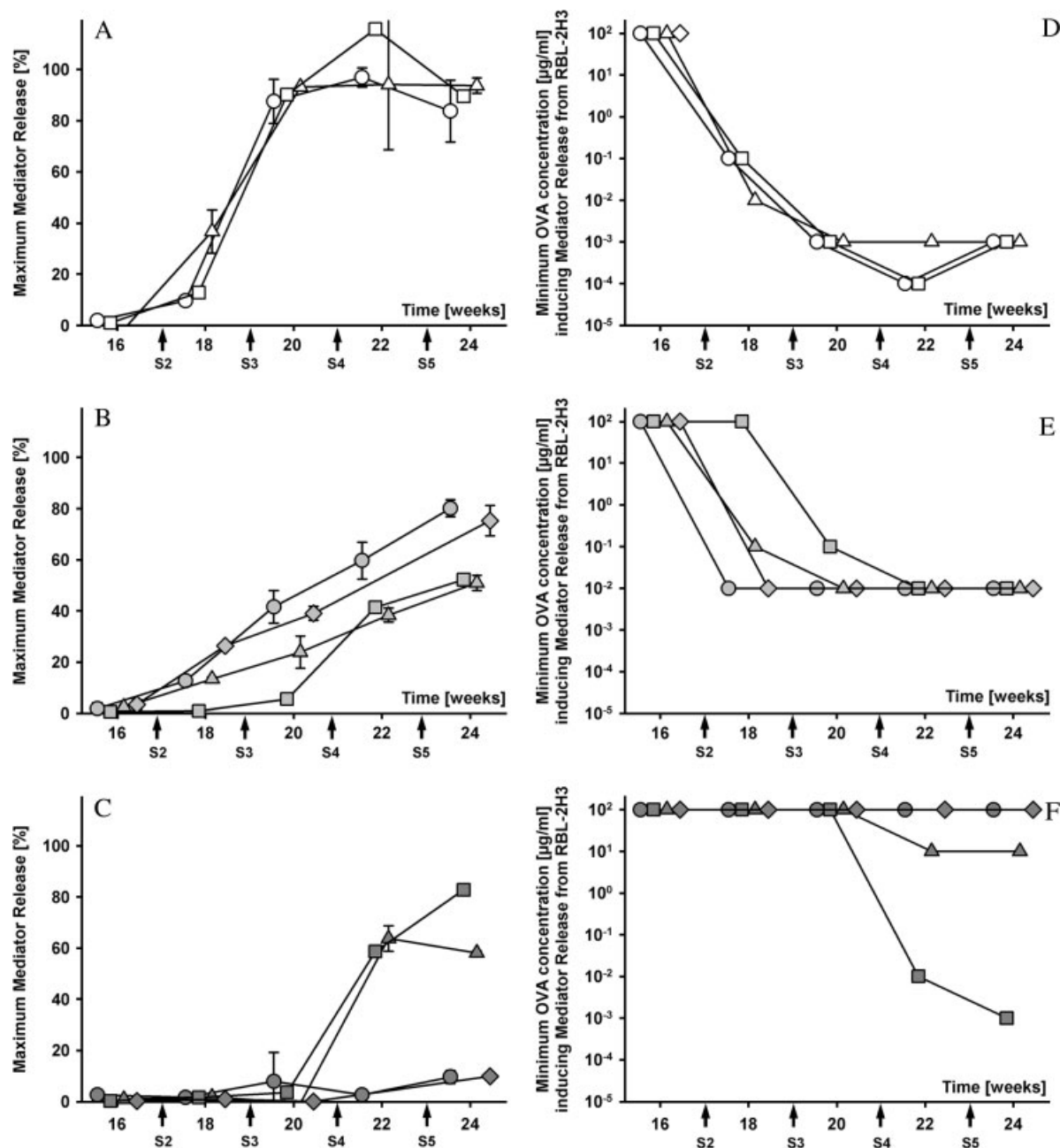


Figure 4. Dose-dependent effect of vaccination on IgE-production. OVA-specific IgE in individual sera from (A, D) unvaccinated mice, (B, E) mice vaccinated with 10^6 IU MVA-OVA or (C, F) 10^8 IU MVA-OVA were measured by RBL assay at different time points (different symbols reflect data from different individual sera). Results were shown as the maximal IgE-mediated mediator release obtained in the test (A–C; reflecting biological activity of specific IgE) and the lowest test concentration of OVA able to induce mediator release in RBL-2H3 cells (D–F; reflecting the degree of sensitization of the animals). Time points of intraperitoneal sensitizations with OVA plus alum are indicated by arrows

sensitivity [37]. Analysis of the IgE response in individual sera revealed that all unvaccinated mice produced high amounts of OVA-specific IgE starting early in the sensitization period. Determination of the minimum stimulation concentration required for a release in RBL assay (minimum stimulation concentration; Figure 4D) showed a decrease that correlated well with the increase of sensitization (maximum mediator release; Figure 4A). Vaccination with 10^6 IU MVA-OVA resulted in alleviated rise of IgE (Figure 4B) and the minimum stimulation concentration dropped only to intermediate level (Figure 4E). By contrast, 50% of mice vaccinated with the high dosage (10^8 IU) did not produce OVA-specific IgE at all and the onset of IgE response in the remaining mice was clearly delayed compared to unvaccinated mice (Figure 4C). Statistical analysis of the data by comparing the areas under the curve of the maximum mediator release curves (percentage values) for each individual between treatment groups by *F*-test demonstrated that the differences between the unvaccinated group and the vaccinated groups are statistically significant (unvaccinated versus 10^6 IU MVA-OVA: $p = 0.0019$ unvaccinated versus 10^8 IU MVA-OVA: $p = 0.0002$) comparisons with a *p*-value below the

significance level of $\alpha = 0.05$ are considered statistically significant, no α -adjustment was performed for multiple comparisons). Moreover, investigation of the minimum stimulation concentration in this group showed that only 25% reached values in the range those from unvaccinated mice (Figure 4F).

Modulation of IgG subclass response by MVA-OVA vaccination

Because the pattern of IgG subclass titers are known to reflect the bias of the T-helper cell response, OVA-specific IgG subclass titers were measured in individual sera from unvaccinated and vaccinated mice. Mice vaccinated with wtMVA without subsequent sensitization did not produce any OVA-specific IgG antibodies.

The two vaccinations in the first 4 weeks were sufficient to elicit a medium level IgG1 titer of approximately 10^6 , which did not change considerably until the first sensitization with OVA (Figure 5A). The following sensitizations led to a continuous increase of the IgG1 titer resulting in a final titer of 10^9 . Although the unvaccinated mice produced OVA-specific IgG1 within 1 week of the

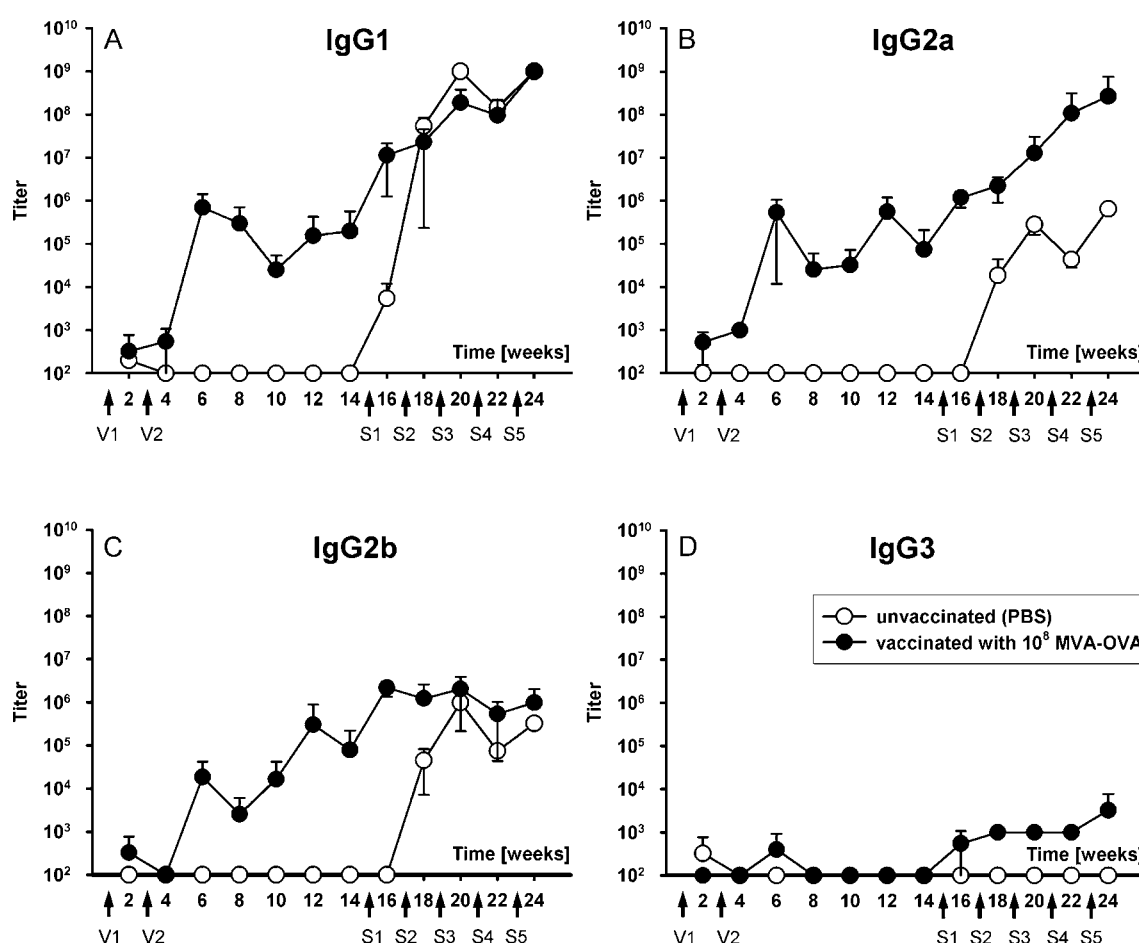


Figure 5. Analysis of OVA-specific IgG subclass antibodies in sera of vaccinated mice. OVA-specific (A) IgG1, (B) IgG2a, (C) IgG2b and (D) IgG3 were determined by ELISA at different time points during the experiment. Thick arrows indicate vaccinations with MVA-OVA. Mice were vaccinated with 10^8 IU MVA-OVA (black circles), control mice (open circles) received PBS. Both groups were sensitized with $0.1 \mu\text{g}$ of OVA at the indicated time points (thin arrows)

first sensitization with OVA, the ascent of the IgG1 titer was much stronger than that in vaccinated mice. However, the IgG1 titers of all mice were similar after the second sensitization showing that, in the present study, high IgG1 titers do not necessarily reflect allergic sensitization. In the sera of vaccinated mice, a constant increase of antigen-specific IgG2a starting after the very first vaccination was observed (Figure 5B). In contrast to the development of IgG1 antibody responses, the IgG2a antibodies in unvaccinated mice never reached the level of those measured in vaccinated mice, with the latter showing at least 100-fold higher titers. This difference may reflect the distinct quality of OVA-specific T-helper responses elicited by vaccination with MVA-OVA. The kinetics of OVA-specific IgG2b titers in unvaccinated and vaccinated mice were similar to that of IgG1 titers; however, the overall titers were lower only reaching 10^6 (Figure 5C) at the end of the experiment. Because IgG3 is thought to be related to a Th1 biased immune response, it could be expected to appear in all groups treated with the MVA-OVA vaccine. Interestingly, OVA-specific IgG3 was only observed in those mice that were vaccinated with MVA-OVA and subsequently sensitized with OVA (Figure 5D).

Vaccination with MVA-OVA gave rise to long-lasting IgG1 and IgG2a titers

The experiments took place over an extended period of 24 weeks, which is long compared to most other known MVA vaccination studies in mice analyzing responses a few weeks after immunization. Indeed, there were no data available predicting on the kinetics and longevity of OVA-specific antibody responses induced by recombinant MVA. To test whether two vaccinations in the first 4 weeks are sufficient to induce a long-term immune response, we investigated the induction of OVA-specific IgG1 and IgG2a antibodies in mice being vaccinated with MVA-OVA but not undergoing subsequent sensitization.

In both groups receiving 10^6 and 10^8 IU MVA-OVA, respectively, a strong increase of IgG1 was detected after the second vaccination (Figure 6A). Interestingly, only minor differences were found in the induction of IgG1 antibodies for the 100-fold difference in the dose of MVA vaccine. Overall, the titers settled down at approximately 10^6 , remained at similar levels at least until week 20 and appeared to moderately decline in the end of the observation period.

The onset of the OVA-specific IgG2a-response appeared somewhat more dose-dependent with 10^8 IU MVA-OVA inducing less than ten-fold higher antibody titers compared to the 10^6 dosage until week 10 of the experiment. Yet, in both groups, the levels of antibodies reached a close to identical maximum titer of approximately 10^6 at week 12 (Figure 6B). Thereafter, as for IgG1, the IgG2a titers were maintained in both groups at approximately 5×10^5 until week 24. These data indicate that vaccinations with recombinant MVA

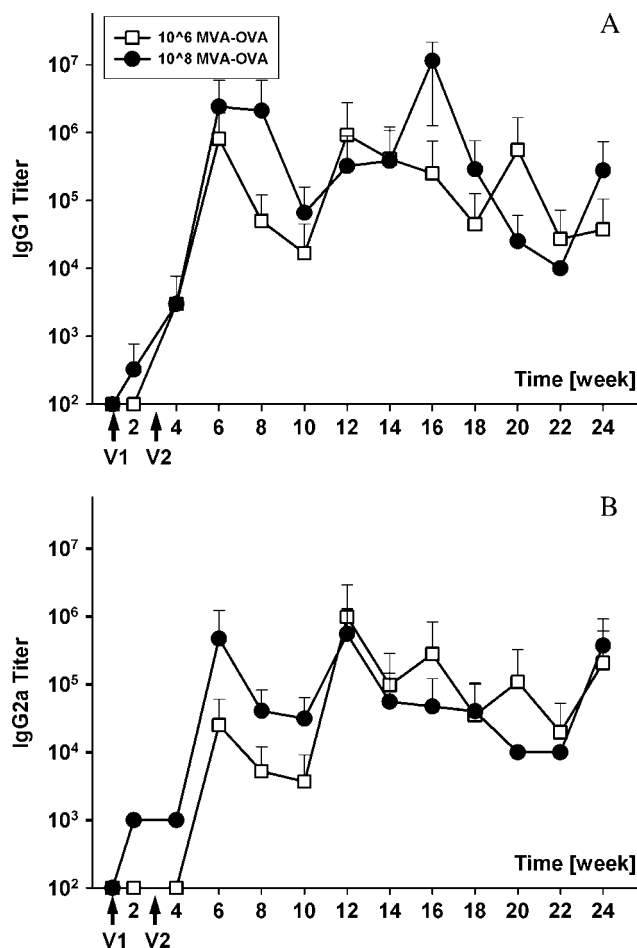


Figure 6. Duration of OVA-specific IgG-response after vaccination without subsequent sensitization. OVA-specific (A) IgG1-titers and (B) IgG2a-titers in individual sera of vaccinated mice (10^6 IU MVA-OVA: open rectangle; 10^8 IU MVA-OVA: black circle), which were not sensitized, were determined by ELISA at different time points after vaccination (indicated with arrows). Values are shown as mean $\pm$ SD ($n = 3-4$)

resulted in a potent acute but also memory immune response that endured for a long time without further antigen stimulation.

Discussion

The classic allergen-specific immunotherapy (SIT) (i.e. injection of increasing allergen doses) is an effective therapy for allergy against pollen and bee venom allergens and therefore application of the allergen itself has tolerizing effects, but it is not applicable in case of food allergy because of the unacceptable high risk of severe adverse systemic reactions [5,6]. For this reason, novel procedures for SIT to food allergy are needed. However, because the 'poison is the cure', the risk of adverse reactions, including potentially fatal anaphylactic shock, is always a possibility. The intracellular virus-driven expression of the allergen by the MVA vaccine described in the present study is a promising approach to improve the safety of allergen immunotherapy.

Only few approaches to allergy therapy employ viruses for SIT of allergies. Mostly, the virus or viral components function as a vehicle to transport DNA efficiently into target cells. Application of virus-like particles (VLPs) carrying a peptide of the major allergen Der p 1 from house dust mite led to strong allergen-specific antibody responses in healthy adults, suggesting that VLPs may be a beneficial adjuvant for SIT [38]. The use of replicon-based DNA vaccines to enhance the immunogenicity of the antigen, and to decrease the high amounts of DNA usually necessary for conventional DNA vaccination, abrogated IgE-production [39,40]. Besides, the MVA-based vaccine investigated in the present study (in contrast to these just mentioned systems) is based on strictly virus-driven synthesis and amplification of the recombinant allergen, which may induce a different and probably more potent antigen-specific immune response relying on the induction of cellular defense mechanisms by the virus.

In the present study, the efficacy of allergen *in vivo*-synthesis by a recombinant MVA-OVA vaccine in preventing allergic sensitization to OVA was determined to investigate the usefulness of this principle for further studies aiming on SIT. Despite the strong IgE-inducing sensitization regimen (five times OVA plus alum), analysis of OVA-specific IgE and IgG subclass responses of vaccinated and unvaccinated mice revealed a remarkable delay in the onset of allergic sensitization, depending on the applied dose of MVA-OVA. Importantly, the analysis of sera obtained from the four additional experimental settings using vaccinations with 10^6 and 10^8 IU MVA-OVA and sensitizations with 1 μ g and 10 μ g of OVA generated almost identical results (data not shown) and robustly confirmed our findings upon sensitization with 0.1 μ g of OVA.

In the high-dose group (10^8 IU MVA-OVA), all mice were protected until the third sensitization and did not produce OVA-specific IgE. The accumulated Th2 type immunogenic pressure of the fourth sensitization appeared to overcome the protective effect of the vaccination. Interestingly, 50% in that particular group did not produce any OVA-specific IgE antibodies by the end of the study, whereas the others did. Such a discrepancy was not observed in the other two groups that received 10^6 IU MVA-OVA, or did not receive any vaccination at all. The diverging OVA-specific IgE responses in the high-dose group may be an indication for a turning point in the immune response, where the decision is made between transient and full protection due to a definitely Th1-biased response.

By contrast to the clear dose-dependent effect observed with the MVA-OVA vaccine, concerning sensitization, a difference in the OVA-specific IgE response was only seen by application of 0.1 μ g of OVA (highest response), whereas delivery of doses of 1 μ g and 10 μ g induced similar sensitization levels, although differing ten-fold in magnitude from the 0.1 μ g dose. This discrepancy between the effects of sensitization and the vaccination vector is probably due to the differing allergen application methods (systemic application or

intracellular expression), thus likely delivering lower amounts of allergen by intracellular expression than by systemic application of 1 μ g or 10 μ g probably reaching a saturation level.

Analysis of the IgG subclass titers clearly revealed a strong tendency towards Th1-like responses in mice vaccinated with MVA-OVA; OVA-specific IgG2a and IgG3 titers were higher than in unvaccinated mice.

The high IgG2a/IgG1 ratios in the vaccinated groups support the notion that the strong Th1-biased immune response elicited by vaccination with MVA-OVA is capable of preventing the isotype switch to IgE. Whether the inhibition of class switch is caused directly by the cytokine milieu of Th1 cells or indirectly by the lack of Th2 secreted cytokines due to the interaction of regulatory T cells remains to be investigated. Regulatory T cells are more likely to be the actors in the mechanisms underlying tolerance to allergens than Th1 polarized cells [41,42].

As mentioned above, one would expect to find IgG3, which is linked to Th1 biased immune responses in all groups treated with MVA. However, OVA specific IgG3 was exclusively found in sera of mice that were vaccinated with MVA-OVA and sensitized with OVA but not in sera of mice that were 'vaccinated' with wtMVA. This raises the question of whether allergen-specific IgG3 might be induced by the particular combination of OVA, the immunomodulator MVA and the adjuvant alum. IgG3 antibodies, which represent the smallest portion of IgG subclass antibodies in mouse serum, are known to be stimulated by T cell independent antigens, such as polysaccharides, and are bound solely by the Fc γ 3RI [43–45]. The description of IgG3 forming self-associating complexes and cryoglobulins connects the accumulation of IgG3 with autoimmune diseases such as lupus erythematosus [46,47]. Moreover, IgG3 production was reported to be induced by interferon- γ , a cytokine that is predominantly secreted by Th1 cells [48]. This could explain the presence of IgG3 in our model because a Th1 biased immune response was observed, but does not answer the question why it did not occur in groups treated with MVA-OVA alone. Possibly, the MVA-OVA primed Th1 biased immune response is amplified by the sensitization process resulting in measurable IgG3 levels. The fact that IgG3 is associated with autoimmune diseases might lead to the assumption that a therapy with a MVA-based vaccine could cause side-effects resulting from a Th1 over-reaction. However, to date, no serious adverse events were observed in all clinical phase I and phase II studies performed with MVA-based vaccines [17,19,23,24,49].

It is surprising that the potential usefulness of rMVA in prophylaxis or therapy of allergic disease has so far not been assessed. The advantage of MVA as a vaccine in SIT lies, among other factors, in its strong and long-lasting immunomodulatory capacity. The duration of stable OVA-specific IgG antibodies titers for at least 24 weeks in mice that received only two vaccinations with MVA-OVA and no further stimulation, underlines the long-term effect of the vaccination. Such a long-term antigen-specific antibody response was also reported for another rMVA [50].

Thus, different rMVA appear to be capable of inducing a memory B-cell response specific for different recombinant antigens, a feature which might be important in terms of prophylactic vaccination with allergen-encoding MVA vector vaccines.

Elucidation of the immunological mechanisms underlying the observed effects by detailed analysis of the immune response will lead to new insights in the properties of preventive immunotherapy. The fact that experiments with a replication-deficient recombinant adenovirus in a therapeutic setting of type I allergy failed to manipulate the Th2 immune response reveals the difficulties of this approach [51]. However, there are several differences between adenoviral vectors and MVA, including the packaging capacity for foreign DNA (approximately 8 kb for adenovirus and 30 kb for MVA) and the replication site (adenoviruses replicate in the nucleus whereas MVAs replicate in the cytoplasm). The most important difference in regard to their usability as prophylactic and therapeutic allergen vaccines may be the possibility of repeatedly applying an MVA vector vaccine to induce a strong antigen-specific boosting effect (Figure 6), whereas adenoviral vectors appear to induce such dominant vector-specific immunity to make repeated administration of the same vector impossible. Lastly, because SIT is known not to abrogate IgE synthesis, in contrast to prevention, a model for therapy of food allergy should include an analysis of symptoms.

Taken together, the presented results demonstrate the capabilities of MVA-based vaccines in the prevention of allergy. One could argue that injection of OVA would have led to the same tolerizing effect, which would very likely be the case. However, in this proof-of-principle study, we wanted to demonstrate that such an effect can be achieved by endogenous allergen expression avoiding the risk of anaphylactic side-effects.

Food allergy models reflecting the intragastric route of sensitization and allergic symptoms are definitely required to investigate the efficacy of a vaccine for SIT. A therapeutic effect of MVA-based vaccines in a (murine) allergy model would suggest rMVA as a novel and promising candidate vaccine for treatment of food allergy. Moreover, MVA is proven to be safe and provides the possibility of applying the vaccine to individuals with a high risk of anaphylactic reactions. MVA-based vaccine may also be used for treatment of allergies to aeroallergens (e.g. house dust mite, grass pollen, tree pollen) and hymenoptera venoms (e.g. bee venom).

Acknowledgements

The authors thank Sandra Krause (Division of Virology, Paul-Ehrlich Institut, Langen, Germany), Stefanie Randow, Sabine Falk, and Andrea Wangorsch (Division of Allergology, Paul-Ehrlich Institut, Langen, Germany) for technical assistance. Furthermore, Kay-Martin Hanschmann (Division for EU Cooperation and Microbiology, Paul-Ehrlich Institut) is acknowledged for the statistical analysis. Funding: Paul-Ehrlich-Institut, European Commission (LSHB-2005-018680, LSHP CT-2006-037536).

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