

Prophylactic mRNA vaccination against allergy

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Purpose of review

mRNA vaccines have recently been re-discovered as an attractive alternative to the more prominent DNA vaccines, as they harbor many advantages with respect to safety and regulatory issues. Whereas most mRNA vaccines are focused on tumor therapy, this type of vaccine has now also been successfully employed for prophylactic immunization against type I allergy in a mouse model. This concept differs from conventional immunotherapy in that it relies on immune deviation toward a TH1 phenotype, rather than induction of regulatory T cells or tolerance.

Recent findings

Conventional as well as self-replicating mRNA vaccines have demonstrated their potential to prevent the induction of an allergic phenotype in terms of allergen-specific IgE, allergy-associated cytokine profiles, eosinophilic lung infiltration, and airway hyperreactivity. Preliminary data raise the question whether TH1 immune deviation induced by mRNA vaccination resembles the natural phenotype of a certain proportion of nonatopic individuals.

Reservations regarding Good Manufacturing Practices manufacture costs, shelf life stability, and lack of immunogenicity due to rapid in-vivo degradation have been overcome by novel findings.

Summary

mRNA vaccines open the field for a safety-optimized prophylactic vaccination against allergic diseases. Future studies concerning long-term effects and vaccine-induced versus natural immune responses will be needed to transfer this knowledge to the clinics.

Keywords

allergy, immunotherapy, mRNA, vaccine

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Introduction

In the past two decades, the high and still increasing incidence of allergic diseases revealed the urgent requirement for alternatives to the existing therapeutic approaches. At present, prophylactic vaccination of children with hereditary risk to develop allergies is seriously considered. Prediction of the risk for sensitization to inhalant allergens is nowadays not solely based on family history and occurrence of food allergies early in life, but also on certain genetic polymorphisms [1,2,3]. Due to further improvement of risk assessment in infants, prophylactic vaccination against allergic diseases has come within reach.

Allergen extracts and recombinant proteins currently used in specific immunotherapy will most likely not be applicable for prophylactic intervention without strong

adjuvantation [4]. Alternatively, immunization with allergen-encoding plasmid DNA has emerged as a promising method for prophylactic and in some cases also therapeutic intervention against type I allergic diseases in various animal models [5,6]. However, due to inherent features of plasmid DNA, some theoretical but in principle possible safety risks are still under discussion, including integration into the host genome [7,8] or induction of pathogenic anti-DNA antibodies due to long-term expression. Hence, plasmid DNA vaccines are classified as gene therapy approaches by the Food and Drug Administration (FDA) in the USA and the Paul Ehrlich Institute in Germany, whereas interventions based on nonreplicative mRNA are not. Given the high safety profile necessary for prophylactic vaccination of children, mRNA vaccines, therefore, represent the logical alternative to plasmid DNA.

Since the first report that injection of naked mRNA encoding a reporter molecule results in antigen expression [9], this approach was employed to trigger protective antitumor immunity [10], to induce humoral and cellular responses after application in the ear pinna either using naked or protected mRNA [11], and to protect mice against a tumor challenge by intradermal injection of total RNA extracted from the respective tumor cell line [12]. Only recently, intradermal injection of autologous preparations of copy mRNA together with granulocyte-macrophage colony-stimulating factor (GM-CSF) into melanoma patients was demonstrated to be safe and to lead to an increase in antitumor humoral responses in some patients [13]. In a second phase I/II clinical trial, protamine-stabilized mRNAs coding for several tumor-associated antigens together with GM-CSF as adjuvant were intradermally injected in metastatic melanoma patients with encouraging results [14[•]]. These data from tumor models confirmed efficacy and safety of mRNA vaccines, making them highly interesting candidates for prophylactic vaccination against allergy.

Types of mRNA vaccine vectors and their immunostimulatory capacities

Conventional mRNA vaccines in general consist of up to five significant portions: the cap structure, the 5'untranslated region (5'UTR), an open reading frame, the 3'untranslated region (3'UTR), and a tail of at least 30 adenosine residues (poly(A) tail). The cap structure and the poly(A) tail influence translation and stability of mRNA vaccines and are essential for effective antigen translation [15,16]. 5' and 3' UTRs form complex interactions with RNA-binding proteins that can result in increased as well as decreased stability and translation efficacy of mRNA. A summary of known UTR subsets and their functions has been recently published [17[•]].

Self-replicating mRNAs represent a different type of mRNA vaccines. In this case, a target antigen is expressed by a bi-cistronic replicative RNA, which encodes both the antigen and an alphavirus-derived RNA replicase, thereby utilizing the ability of alphaviruses to produce large amounts of viral mRNA. Following transfection of a cell, the viral RNA is amplified by the replicase complex, which synthesizes a genomic negative strand that itself serves as a template for the synthesis of numerous genomic RNA-positive strands by the replicase. Subsequently, the replicase drives the transcription of capped mRNA from a subgenomic promoter [18].

The superior immunostimulatory capacity of self-replicating nucleic vaccines seems to result from both high amounts of antigen produced and nonspecific immunostimulation via double-stranded RNA intermediates formed during RNA replication, which activate anti-

gen-presenting cells through Toll-like receptor 3 (TLR3) and induce immune apoptosis [19]. It has been shown that induction of type I interferons is essential for the immunogenicity of replicase-based vaccines [20]; however, recent evidence shows that other sensors of dsRNA, such as the RNA helicase encoded by retinoic acid inducible gene I (RIG-I) [21], can compensate for TLR3 [22]. Stabilized mRNA has been described as a type of immunostimulating RNA, which differs from synthetic double-stranded RNA with respect to its immunostimulation profile [23] in that it activates TLR7 in mice [24] and TLR8 in humans [25]. Although TLR recognition of ssRNA and dsRNA definitely boosts immunogenicity, the fact that both types of RNA vaccines elicit – though reduced – immune responses in MyD88^{-/-}-knockout mice indicates that alternative pathways exist [26[•]].

Pharmacological properties and stability of RNA vaccines

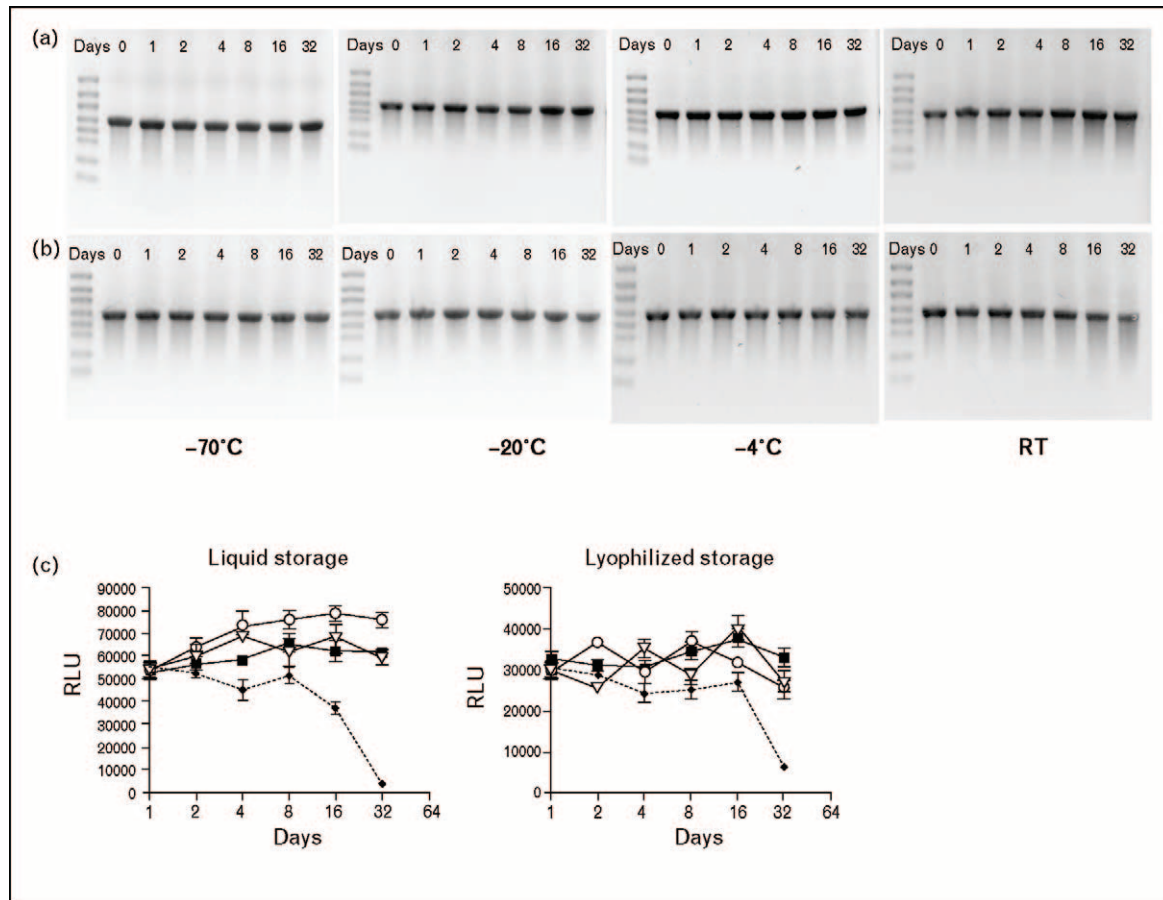
Manipulation as well as purification of mRNAs is rather simple. Starting from a linearized plasmid DNA template, mRNA is transcribed *in vitro* from a bacteriophage polymerase promoter (T3, T7, or SP6) followed by DNase-mediated degradation of template DNA. The 5' cap structure is generated by either incorporating cap analogues [15] during transcription or by a second enzymatic step using vaccinia virus capping enzyme [27]. RNA can then be recovered by LiCl or (NH₄)₂SO₄ precipitation. For GMP production, mRNA is further purified by chromatographic methods to eliminate shorter or longer transcripts.

Surprisingly, stability of pure mRNA turned out to be no major issue because in the absence of contaminating RNases, mRNA is highly stable. Even under standard laboratory conditions, without the use of any RNase inhibitors, RNA vaccines are stable and maintain their full translation capacity even at higher storage temperatures (Fig. 1).

RNA vaccines prepared under GMP conditions can be expected to display even higher stability and extended shelf life. Compared with DNA, pharmaceutical safety of mRNA is superior because it does not integrate into the genome and the transfection remains transient, as once injected into the body, mRNA is rapidly degraded by ubiquitous RNases [26[•],28,29].

Uptake and intracellular transport of RNA vaccines

The mechanisms of intracellular uptake and trafficking of naked nucleic acid vaccines are poorly understood. Most studies have been performed with plasmid DNA;

Figure 1 Stability of RNA preparations

mRNA encoding luciferase was stored for 32 days at the indicated temperatures either in water (a) or lyophilized (b). Integrity of RNA was assessed via denaturing agarose gel electrophoresis (a and b) and luciferase expression in transfected BHK-21 cells (c). RT, room temperature. Reproduced from [26]. ■, -70°C; ○, -20°C; ▽, +4°C; ♦, RT.

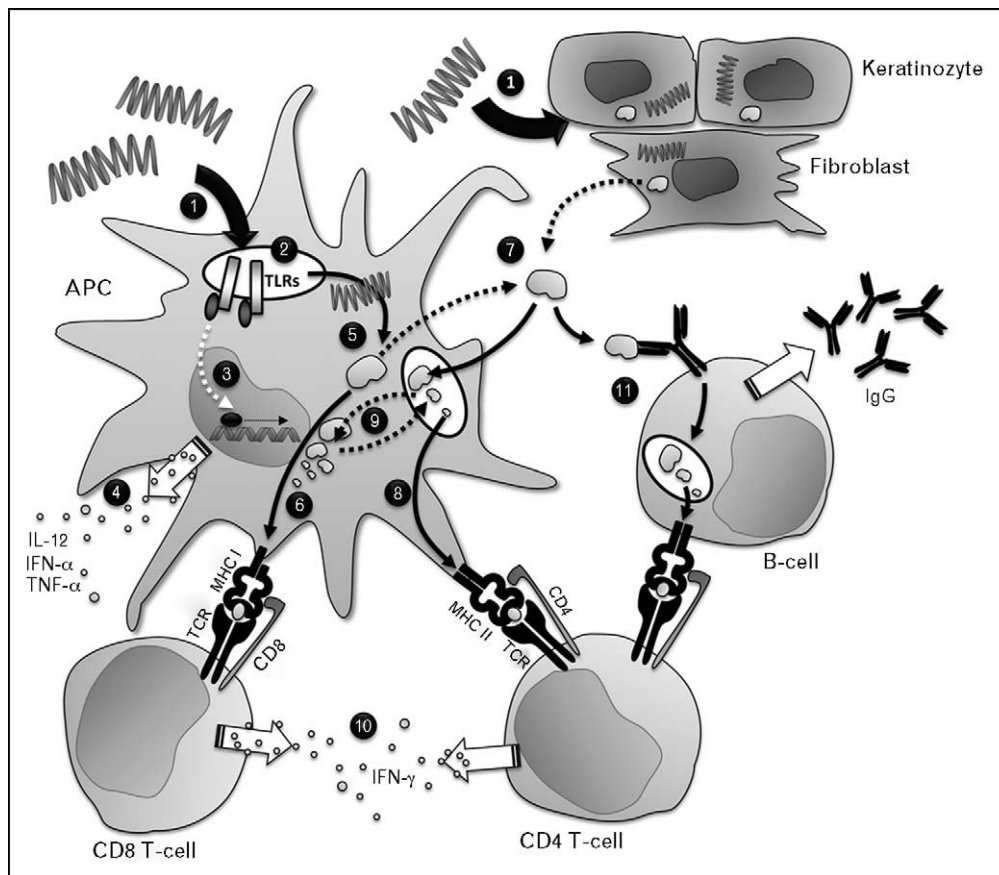
however, some general features can be expected to be applicable for RNA vaccines as well. Some publications have suggested that naked DNA can be taken up by muscle cells through caveolae [30,31], whereas in contrast, keratinocytes take up plasmids via macropinocytosis [32]. In another study, clathrin-coated pits seem to be involved in cellular uptake by macrophages [33]. Concerning the uptake of mRNA, a recent publication demonstrated that exogenous RNA is going through moving vesicles before being delivered to the cytosol, but inhibitors blocking macropinocytosis, clathrin-coated pits, or caveolae showed no effect [29]. Evidence exists that DNA and RNA uptake is receptor-mediated and dependent on the negative charge of the molecules. Several DNA-binding proteins have been identified in various cell types [32,34,35]. Indeed, in-vivo uptake of mRNA after intradermal injection was a saturable process that could be blocked by adding an excess of any type of nucleic acid but not peptide [29]. In *Drosophila*, uptake of dsRNA seems to be mediated by members of the scavenger receptor family and is dependent on the length

of the RNA molecule. Interestingly, dsRNA was taken up much faster compared with DNA of the same length [36]. Most likely, uptake of nucleic acids in general includes several receptors simultaneously and may also be sequence-specific [37,38].

Little is known about how DNA/RNA vaccines escape from endosomes into the cytoplasm of the cell. A recent study [33] suggests that plasmid DNA emigrates from early endosomes into vesicles that transport DNA from the early endosome to the nucleus, thereby protecting the DNA during its transport from the endosome to the nuclear membrane against rapid degradation by cytosolic DNases [39]. However, at least in *Drosophila* cells, there seem to be different kinetics and subcellular distribution of dsRNA compared with DNA after the uptake, indicating that differences in the vesicular transport and potential endosomal escape may exist between RNA and DNA [36].

In contrast to DNA vaccines, mRNA vaccines exert their function in the cytoplasm of the cell, so they need not

Figure 2 Basic principles of RNA vaccination



(1) Naked RNA vaccines injected intradermally can directly transfect skin resident antigen-presenting cells (APCs) or somatic cells such as keratinocytes and fibroblasts. (2) In the endosome, Toll-like receptors (TLRs) 7 and 8 (ssRNA) or TLR3 (dsRNA) can be stimulated by RNA vaccines resulting in activation of nuclear transcription factors such as activator protein 1 (AP-1), nuclear factor- κ B (NF- κ B), or interferon-stimulated response element (ISRE) (3). These factors cause transcription and secretion of several cytokines, including IL-12, interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α) (4). After endosomal escape, mRNA is translated into the corresponding protein (5) that can be degraded into peptides that in turn are presented on major histocompatibility complex (MHC) class I (6). Depending on the nature of the protein and the presence of suitable signal sequences, translated protein will also be secreted from transfected cells (7) and can then be re-acquired by APCs resulting in presentation on MHC class II (8). Alternatively, peptides from the endogenous pathway may be presented on MHC II via autophagy and peptides from the exogenous pathway on MHC I via cross-presentation (9). Presentation of peptides by IL-12-secreting APCs results in the activation of cytotoxic T cells and type-I T helper cells, which in turn secrete IFN- γ (10). Secreted protein activates antigen-specific B cells (11) to produce IgG antibodies.

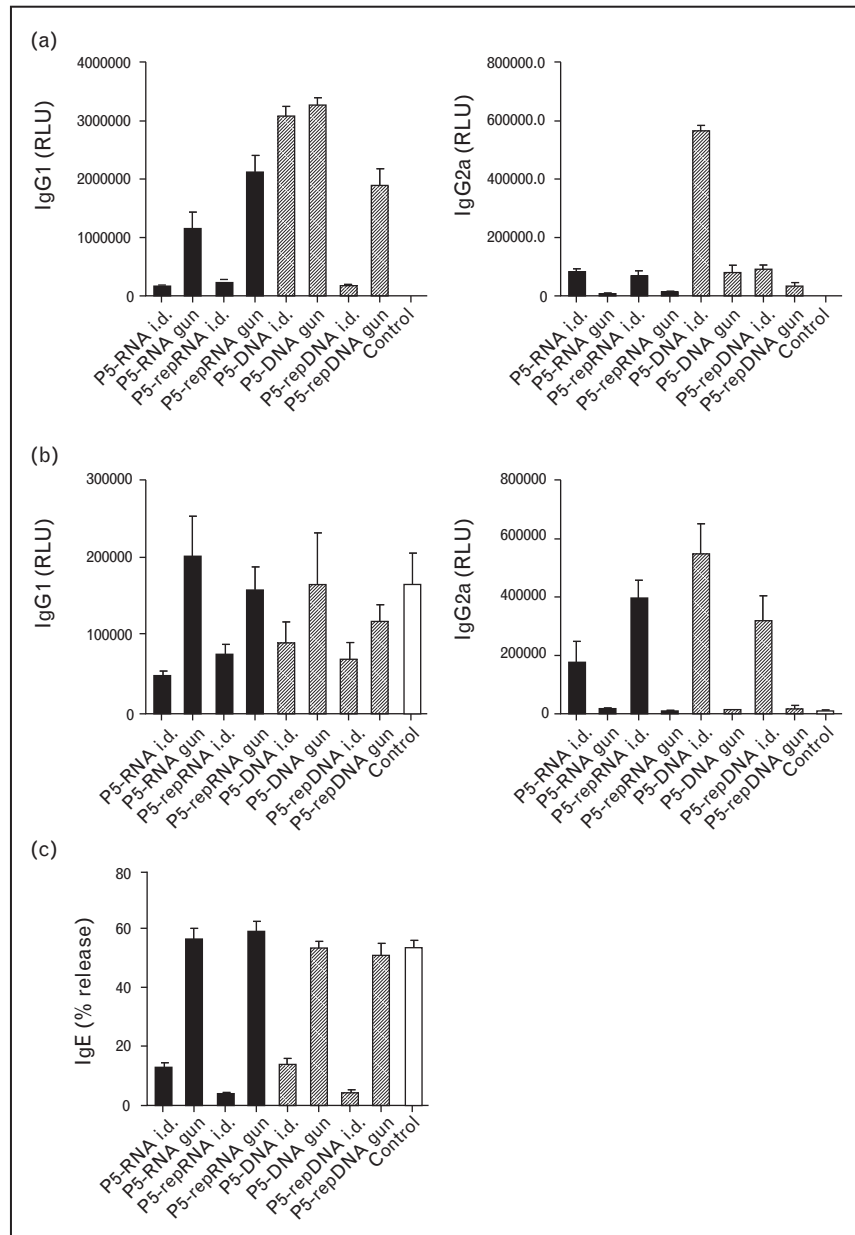
cross the nuclear membrane, thus resulting in a higher transfection capacity, especially in nondividing cells [40,41]. Consequently, mRNA vaccination induces a higher peak expression compared with plasmid DNA after intradermal injection [16,26*]. The basic principles of mRNA immunization are depicted in Fig. 2.

Prophylactic mRNA vaccination against allergy

We and others have previously shown that the protective capacity of antiallergic DNA vaccines is clearly associated with the induction of interferon- γ (IFN- γ)-secreting T cells [42]. The first studies demonstrating the induction of a specific immune response by mRNA indicated that the induced immune response was of a TH2-type, which

could only be reverted into TH1 by addition of GM-CSF as adjuvant. Within this context, it was surprising for us that indeed RNA vaccines exerted similar protective effects against allergic sensitization compared to DNA vaccines.

When we compared the immunogenicity of conventional as well as self-replicating RNA and DNA vaccines encoding the grass pollen allergen Phl p 5, we observed weaker antibody responses in the RNA vaccine groups compared with the DNA vaccine groups (Fig. 3a). However, after sensitization with recombinant allergen adjuvanted with alum, intradermal priming with RNA vaccines resulted in a similar boost of IgG2a as compared to DNA vaccines (Fig. 3b), indicating that although the primary immune reaction may be weaker for RNA vaccines, the initially

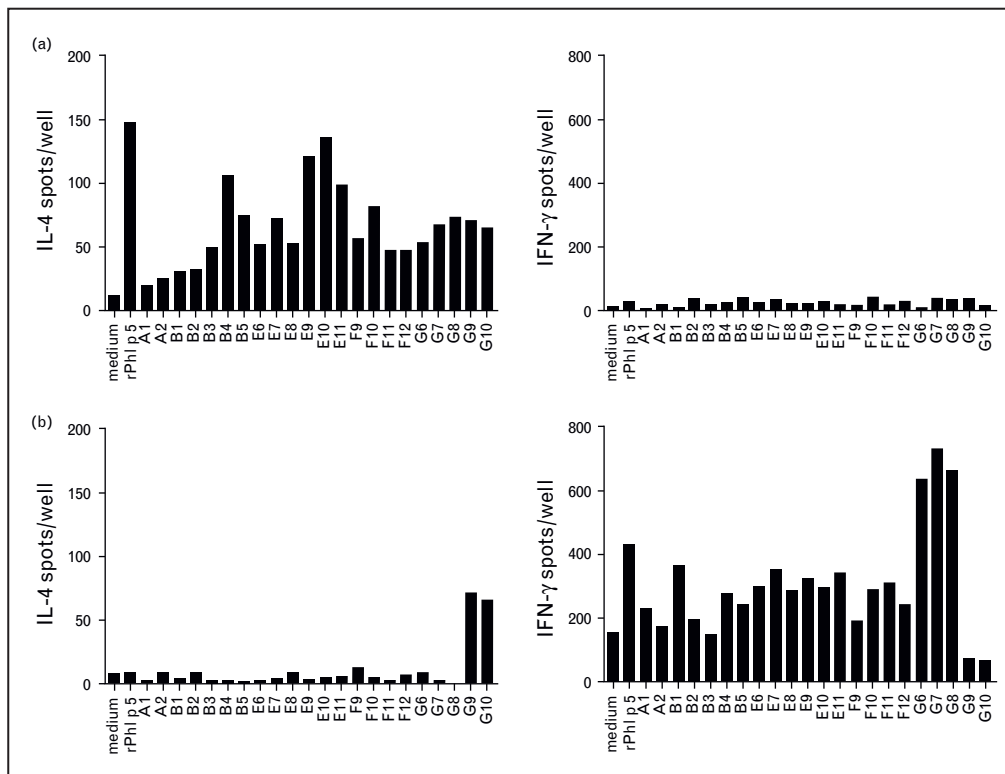
Figure 3 mRNA vaccines protect from allergic sensitization

Immune responses after vaccination with RNA (solid bars) and DNA (hatched bars) vaccines encoding Phl p 5 (P5-RNA, P5-DNA) and their self-replicating counterparts (P5-repRNA, P5-repDNA) were compared. Mice were immunized three times in weekly intervals by intradermal injection or via gene gun (gun). Sensitization controls (control, open bars) were naive prior to sensitization. (a) Phl p 5-specific IgG1 and IgG2a after the 3rd vaccination. (b) IgG1 and IgG2a levels of the corresponding groups after a subsequent challenge using two subcutaneous injections with recombinant allergen in alum. (c) Allergen-specific IgE after sensitization. Data are shown as means $\pm$ SEM ($n = 4$) of relative light units (RLU) of a luminescence-based ELISA assay (a and b) or mediator release in a basophil release assay (c). Serum dilutions were 1 : 1000 (a), 1 : 100 000 (b), and 1 : 100 (c).

set TH1 bias is expanded by a concomitant challenge with protein/alum similar to what we see for DNA vaccines. Consequently, these mice were protected from induction of allergen-specific IgE (Fig. 3c) and protection from IgE strongly correlated with induction of IgG2a after sensitization (Fig. 3b, right graph). Gene gun vac-

cination with RNA vaccines had no protective effect from allergic sensitization (Fig. 3c), which is in agreement with previous results with DNA vaccines [43].

Further analysis of the antiallergic potential of Phl p 5 encoding RNA vaccines revealed that prophylactic

Figure 4 Allergen-specific response of an atopic and a nonallergic individual

ELISPOT data of a Phl p 5 allergic patient (a) and a nonatopic, healthy individual, who was raised and still lives in a farming environment (b). Peripheral blood mononuclear cells (PBMCs) were expanded by re-stimulation with rPhl p 5 and after a resting period assayed by ELISPOT without stimulation (medium), or re-stimulation with rPhl p 5, or immunodominant peptides resembling class II epitopes of Phl p 5 (A1 – G10).

vaccination not only protected from the induction of IgE, but also downregulated inflammatory lung parameters, including accumulation of eosinophilic granulocytes in bronchoalveolar lavage fluids as well as airway hyperreactivity. Moreover, the broad applicability of this approach was confirmed by analyzing the TH1 induction capacity for a panel of 28 other major pollen, food, animal, mold, and latex allergens [26*].

Prophylactic RNA vaccination versus the natural immune response

A frequently uttered criticism concerning the induction of a protective immune response with RNA or DNA vaccines is that a TH1 bias would not reflect the natural status of a healthy nonatopic immune system. This argumentation is built on the fact that effective gene vaccination against allergy gives no evidence for the induction of IL-10-secreting regulatory T cells, but relies on TH1 immune deviation. The claim that a natural, asymptomatic immune response against an allergen is solely driven by IL-10-secreting Tregs mainly originates from studies on bee keepers regarding phospholipase A [44]. However, the situation concerning environmental

pollen allergens seems to be different and more complex. This is indicated by recent preliminary experiments, in which we performed epitope mapping and IL-4/IFN- γ ELISPOT assays with peripheral blood mononuclear cells (PBMCs) of a Phl p 5 allergic and two nonallergic healthy individuals. As expected, the allergic person mounted strong Phl p 5-specific proliferation with high amounts of IL-4-secreting cells (Fig. 4a). Of the nonatopic individuals, one did not display Phl p 5-specific proliferation and neither IFN- γ nor IL-4 secretion, which obviously reflects the 'tolerant' nonresponsive status of a healthy nonallergic immune system. Interestingly, the second nonatopic individual displayed strong Phl p 5-specific proliferation that was TH1-dominated (Fig. 4b). We think that this may resemble the natural protective immune response of individuals who were exposed to pathogen-associated molecular patterns (PAMPs) during childhood together with pollen allergen.

Despite being preliminary, these results clearly give rise to the assumption that a 'healthy' immune response against allergens in humans need not necessarily be based only on Tregs, but can also be mediated by IFN- γ -secreting T cells, resulting in immune deviation rather

than tolerance induction. This would also be in agreement with many observations from epidemiological studies [45] that found correlations between a decreased risk for allergy and microbial contact and sustained exposition to an allergen over years (e.g. exposition to microbes and hay correlates with decreased risk for grass pollen allergy, personal communication, E. von Mutius). Interestingly, the nonatopic person with a TH1-type response against allergy was raised and still lives in a farming environment.

Taken together, the priming of the TH1 memory by prophylactic RNA vaccination and its subsequent maintenance/boosting by later environmental exposure to allergen might resemble the natural situation of individuals primed and protected in a farming environment.

Conclusion

mRNA vaccines can bias the naive immune system of an individual toward a TH1-type direction, thereby preventing the development of allergic disease. Preliminary data indicate that this subtle priming by mRNA vaccines may resemble the same situation as described by the hygiene hypothesis, when simultaneous exposure to allergens and PAMPs results in the generation of asymptomatic, allergen-specific TH1 memory. Given the higher safety profile of mRNA vaccines compared with DNA vaccines, mRNA vaccines are, therefore, promising candidates for prophylactic vaccination of children with a high hereditary risk to develop allergies. Future experiments have to investigate whether the protective immune deviation is maintained over recurrent (seasonal) allergen exposure or even boosted and enhanced by a later contact with allergen.

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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