A Novel Method to Enhance Immune Responses Induced by HIV DNA Vaccination

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Significance of Work

The original Procedure described in this article represents a breakthrough that has the potential to revolutionize the field of DNA vaccinations in areas as diverse as HIV and cancer. This Procedure is not specific for a particular DNA sequence and could be applied to conditions unrelated to HIV or cancer, where DNA vaccines are under investigation, such as atherosclerosis and Alzheimer’s disease.

Abstract

Reason

HIV DNA vaccination is promising technology, but still not capable of inducing strong immune responses. Novel strategies aimed at improving the effectiveness of DNA vaccines are needed.

Methods

Formation of multi-molecular complexes for enhancing immune responses induced by HIV DNA vaccination is achieved through the sequential interaction of plasmid DNA with poly-L-lysine, microbial chondroitin sulfate, and phosphatidylcholine.

Results

A novel method to enhance immune responses induced by HIV DNA vaccination results in the genesis of a multi-molecular complex. This complex consists of an inner core of plasmid DNA vaccine surrounded by macromolecules, first by poly-L-lysine, then by chondroitin sulfate, finally by phosphatidylcholine. The structure of this multi-molecular complex is superimposable to that of liposomes or micelles.

Conclusion

The Procedure described in this paper represents a novel method to overcome current limitations of DNA vaccination in conditions as diverse as HIV and cancer. In addition, this Procedure is not specific for a particular DNA sequence and could also be applied to conditions unrelated to HIV or cancer where DNA vaccines are under investigation, for example, in atherosclerosis and Alzheimer’s disease.

Key Words: HIV; Vaccine; Chondroitin Sulfate; Poly-L-Lysine; Phosphatidylcholine

Introduction

The search for a vaccine to prevent AIDS dates back to the early days of the AIDS epidemic. After many failed attempts to produce a preventative vaccine, a recent breakthrough may have accomplished the decades-old objective. Researchers at the State Research Institute of Highly Pure Biopreparations, The Biomedical Center of the "Peter the Great" Polytechnic University, and the "Pavlov" State Medical University of Saint Petersburg, Russia published a seminal paper, reporting for the first time, 100% immune responses in a trial using a HIV DNA vaccine [1].

The Russian researchers used a multigene DNA vaccine expressing HIV-1 clade A Nef, Gag, RT and Env proteins. A sterile solution with different amounts of DNA (0.25, 0.5 or 1.0 mg) was injected intramuscularly in 21 healthy HIV-1-negative adult volunteers aged 20–45 years (8 females, 13 males). Four injections were administered (at days 0, 6, 10 and 14) and blood samples were collected for up to 60 days after the first injection. All participants showed positive T-cell mediated immune responses, while only one quarter of participants showed positive humoral responses thus confirming that cell-mediated responses were prevalent.

Despite the excellent results in immunogenicity, the Authors pointed out that the magnitude of the immune responses had to be classified

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as "weak", a limitation common to DNA vaccines. It seemed therefore evident that strategies aimed at enhancing immune responses induced by DNA vaccines would be necessary for the success of DNA vaccination against HIV or for use in other conditions such as cancer [2].

In this paper, I describe a novel molecular system that will perform the task of boosting, or enhancing, immune responses to DNA vaccines, as well as the task of delivering DNA vaccines that might not require injections.

**Description of the Procedure**

The DNA vaccine booster/delivery system described here is based on a multi-molecular arrangement of two macromolecules (chondroitin sulfate and poly-L-lysine) and a phospholipid (phosphatidylcholine) that together encapsulate the plasmid DNA of the vaccine, and described in Akulova et al. [1] as an example of a successful DNA vaccine.

The first step of the Procedure consists of the formation of poly-L-lysine/DNA complexes. This step is conceptually similar to the formation of arginine-rich peptide/DNA complexes as they were described by Naik et al. [3]. Differing with the procedure described by Naik et al., poly-L-lysine is used instead of arginine-rich peptides. There are several reasons that make the use of poly-L-lysine preferable to the use of arginine-rich peptides.

1. Poly-L-lysine is an inexpensive, commonly available food additive generally recognized as safe (GRAS) in the United States.
2. Poly-L-lysine shows a high density of positive charges that interact with negatively charged macromolecules. This interaction forms soluble complexes that can be used to deliver DNA [4].
3. Poly-L-lysine is known to interact with highly sulfated glycosaminoglycans such as heparin and chondroitin sulfate [5]. These interactions replicate the molecular assembly described by Naik et al. for arginine-rich peptide/DNA complexes [3].

In the case of a DNA vaccine, like the vaccine described by Akulova et al. [1], poly-L-lysine/plasmid DNA complexes can be prepared using a 30:1 ratio for positive charges of poly-L-lysine per each negative charge of DNA. Such a ratio is bigger than the ratio described by Naik et al. for arginine-rich peptides (10:1). This understanding derives from the observation that a 20:1 ratio poly-L-lysine/glycosaminoglycan is necessary to neutralize the negative charges on the surface of glycosaminoglycans [5], and a 10:1 ratio poly-L-lysine/DNA is necessary to form complexes with plasmid DNA [3]. Since the first step of the procedure consists in the formation of poly-L-lysine/DNA complexes at room temperature for 1 h, the ratio 30:1 guarantees that the resulting complexes are endowed with a net positive charge and are thus ready for the second step of the procedure that adds a novel form of chondroitin sulfate.

Chondroitin sulfate serves four purposes in this Procedure in producing an innovative DNA vaccine booster/delivery system:

1. Improves intracellular routing and nuclear accumulation of poly-L-lysine/DNA complexes [3].
2. Functions as an adjuvant stimulating the immune system with particular effect on cell-mediated responses [6].
3. Determines the formation of liposomal structures with phosphatidylcholine [7].
4. Exerts direct anti-HIV effects, as well as effectiveness in the immunotherapy of cancer [8,9].

At variance with previous studies, this Procedure does not use chondroitin sulfate of animal origin, but rather a novel type of chondroitin sulfate of microbial origin whose efficacy, safety and human bio-availability have been demonstrated [10]. Chondroitin sulfate of microbial origin offers the advantage of being more potent than pharmaceutical grade chondroitin sulfate of animal origin [11]. In the Procedure described here, low-molecular-mass chondroitin sulfate of microbial origin is incubated with poly-L-lysine/DNA complexes in gently stirring buffered saline for 1 h at 37°C with a 20:1 ratio of poly-L-lysine: chondroitin sulfate. Formation of neutral complexes constituted by low-molecular-mass chondroitin sulfate, poly-L-lysine and plasmid DNA is assessed following the procedure described in Pacini et al. [5].

The third step of the Procedure consists in the formation of natural liposomes thanks to the interaction between phosphatidylcholine and the macromolecular complexes described above, with particular reference to chondroitin sulfate. We have demonstrated since 1985 that glycosaminoglycans circulate in bloodstream associated with phosphatidylcholine, and that the 50:1 ratio of phosphatidylcholine:glycosaminoglycan represents the ideal ratio for formation of liposomal complexes constituted by phosphatidylcholine and glycosaminoglycans [7]. In the Procedure described in this paper, phosphatidylcholine is incubated for 48 h at 37°C with a 50:1 of ratio phosphatidylcholine: chondroitin sulfate following the procedure described in Vannucchi et al. [7].

At the end of the 48 h incubation time, the resulting multi-molecular complex consists of an inner core of plasmid DNA vaccine surrounded by the macromolecule poly-L-lysine associated with chondroitin sulfate. This complex is then surrounded by phosphatidylcholine, thus forming a structure super imposable onto liposomes or micelles.

The fourth step of the Procedure consists in sterilization of the multi-molecular complexes described above, using 100 nM filtration. This Procedure offers the advantage of nano sizing the complexes, thus improving their bio-availability. The significantly increased contact surface area guarantees that a greater number of molecules will interact with the intended biological targets.

Different routes of administration can be utilized with this Procedure to deliver this multi-molecular complex.
1. Parenteral injection [1].
2. Transdermal delivery using pulsed-current iontophoresis as described in Pacini et al. [12].
3. Transdermal delivery using ultrasounds as described in Klinghardt and Ruggiero [13].
4. Oral, sublingual, nasal, mucosal or dermatological administration using appropriately devised matrices aimed at favoring absorption as described, for example, in Rai et al. [14].

Discussion

The Procedure described in this paper represents a breakthrough that has the potential to revolutionize the field of DNA vaccination in areas as different as HIV and cancer. This Procedure exploits approaches that have proven useful in delivering DNA- and protein-based drugs and combinations, in one single solution. This Procedure employs different strategies with the goal of enhancing the efficiency of DNA vaccines in inducing immune responses while providing an ideal delivery system that could be applied in a variety of conditions. In addition, the Procedure enables the transfer of results and concepts acquired in one field of research to other seemingly unrelated fields. For example, Akulova et al. demonstrated that the HIV DNA vaccine designated DNA-4 was able to induce a weak, albeit significant, immune response based on activation of T-cells expressing Tumor Necrosis Factor-alpha (TNF-α) and Interferon-gamma (Ifn-gamma) [1]. It is well accepted that TNF-α promotes both innate and adaptive immune responses and plays a fundamental role in protection against tumor development and the so-called “cancer immunoeediting” [16]. Therefore, it could be argued that the DNA-4 vaccine would be effective as an anti-cancer vaccine provided it was able to induce a stronger response than that observed by Akulova et al. This point raises the interesting question as to whether immunological responses to HIV infection do indeed protect against tumor development and progression.

Such a hypothesis was published by Lulli et al. in 2010 in a paper where “the possibility that HIV might be endowed with anti-tumour activity” is mentioned [17]. Consistent with this statement, studies on the progression of breast cancer in HIV-positive women suggest a level of protection is exerted by the immune response elicited by HIV. A study performed at Columbia University in New York reported that HIV-positive women have better five-year survival rates when compared to a HIV-indeterminate population [18]. Interestingly, the same Authors wrote in a successive study, “Of note is the finding that HIV infection in premenopausal women was not associated with aggressive breast cancer subtypes with poor survival outcome” [19]. These apparently odd results, or outliers at the time, may now be re-interpreted, taking into account the immune responses observed by Akulova et al. [1]: T-cell activation induced by viral antigens leads to the production of immune stimulating factors that may not only protect against progression toward immune deficiency but may also protect against cancer development and aggressiveness.

It is worth noticing, however, that vaccine-induced immune system stimulation may represent a double-edged sword in chronic HIV infection, a condition that is characterized by persistent activation of the immune system by replicating HIV possibly leading to immune exhaustion. In this occurrence, effector T cells become dysfunctional and their effector functions and proliferative capacity are significantly impaired [22]. This phenomenon is peculiar of chronic HIV infection where persistent viral replication leads to aberrant immune activation and cellular exhaustion [23]. It can be argued that this is one of the reasons why current HIV vaccines have not yielded the expected results [1]. The Procedure described in this paper addresses this problem by incorporating in the molecular structure of the DNA vaccine chondroitin sulfate, a glycosaminoglycan that specifically inhibits the binding of HIV glycoprotein gp120 to its host cell CD4 receptor [24], and is intrinsically endowed with anti-HIV activity [8]. Therefore, this Procedure may be considered a fully integrated immunotherapeutic approach where not only is the immune system stimulated by the vaccine, but, equally important, HIV replication is inhibited by chondroitin sulfate, thus preventing immune system exhaustion due to persistent HIV replication.

Conclusion

The Procedure described in this paper represents a novel method for overcoming the current limitations of DNA vaccination in conditions as diverse as HIV and cancer. In addition, since this Procedure is not specific for a particular DNA sequence, it could also be applied in areas unrelated to HIV or cancer where today DNA vaccines are being explored, for example, in atherosclerosis [20] and Alzheimer’s disease [21].

Author’s Contribution

Marco Ruggiero developed the concepts described in this paper and wrote the manuscript.

Competing Interests

Marco Ruggiero is the founder and CEO of Silver Spring, a Swiss research and development company in the field of supplements and probiotics. No products of Silver Spring are mentioned in this study. Marco Ruggiero is the inventor of the immune stimulating molecule designated Rerum mentioned in reference n. 9.

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