

# A trail blazed through DNA vaccine, noninvasive vaccine, and innate-adaptive immunity duo

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## DNA Vaccine: My First Adventure in Vaccinology

I entered the field of vaccinology when I joined Dr. Stephen Johnston's laboratory as a postdoc in 1990. He and Dr. John Sanford co-designed and fabricated the first hand-held gene gun and my job was to transfect live animals by delivering DNA-coated gold particles into a restricted subset of tissues under the gun point. After bombarding a recombinant plasmid encoding a human protein into the outer layer of murine skin, we demonstrated that serum antibodies to the human protein were elicited in transfected mice within a few weeks.<sup>1</sup> This demonstration marked the first chapter of "DNA vaccine"; with the technique originally dubbed "genetic immunization" by Dr. Johnston. A year later, others reported that mice could be immunized following intramuscular injection of DNA with a conventional syringe needle.<sup>2</sup> Comparative studies subsequently showed that gene gun inoculation outperformed intramuscular injection in mobilizing the immune repertoire toward beneficial protection of animals.<sup>3</sup> To circumvent the low immunogenicity associated with needle injection, development of *in vivo* electroporation<sup>4</sup> has provided another technique capable of immunizing animals with DNA vaccines to approximately the same caliber as that achievable by a gene gun.<sup>3,5</sup> To date, human subjects have been effectively immunized following *in situ* DNA delivery by either electroporation<sup>6</sup> or gene gun inoculation.<sup>3,7</sup>

In Dr. Johnston's laboratory, we made an array of serendipitous observations that

could hardly be explained by textbook knowledge. It was demonstrated that mice were effectively immunized when DNA was inoculated into the outermost layer of skin without going deep, as shown by  $\beta$ -galactosidase reporter expression within epidermis one day post-immunization.<sup>8</sup> In conventional wisdom, transfected cutaneous cells (visible transfected cells as shown by reporter expression one day post-immunization were all keratinocytes<sup>8</sup>) should broadcast a signal to recruit antigen-presenting cells (APC) for capturing the exogenous proteins made in transfected cells in conjunction with subsequent antigen presentation to T lymphocytes for eliciting an immune response; thus cross priming<sup>9</sup> may not occur until the exogenous protein is expressed from the man-made DNA *in vivo* which takes approximately one day to reach its peak level.<sup>10</sup> To our surprise, mice (the so-called van Gogh mice) could be effectively immunized when their transfected ear pinnae were surgically removed 5 min after inoculating DNA into the pinnae using a gene gun—an anti-dogmatic observation that potentially may lead research to a terra incognita. We subsequently identified dynamic relocation of discrete reporter spots from the transfected pinna to scalp, neck skin, and skin on the dorsal side; but never found such mobile reporter spots in skin on the ventral side of the animal one day following transfection of ear pinnae with the luciferase reporter gene using a gene gun (unpublished results). Although keratinocytes in an ear pinna could be transfected in large numbers under gun point,<sup>8</sup> evidence implied that keratinocytes in the outermost layer of epidermis may not qualitatively be a

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major player during genetic immunization. However, the mechanisms of action remain unsettled.

Only a decade ago, DNA vaccines were an unproven novelty with limited acceptance in the scientific community even though the advent of DNA vaccines potentially may expedite vaccine production because the often difficult steps of protein purification and combination with adjuvant, both routinely required for vaccine development, are eliminated. Since using DNA as a vaccine does not require the purification of proteins, it is particularly valuable for proteins that may lose conformational epitopes when extracted and purified biochemically. Presumably, endogenous expression of antigens in vaccinees' own cells may play a crucial role in the relatively greater efficacy of DNA vaccine over its protein-based counterpart in eliciting cellular immunity.<sup>11</sup> Moreover, recombinant DNA technology can generate new DNA vaccines rapidly and creatively at low costs without requiring a cold chain for storage and shipment.

To date, several DNA vaccines have been licensed for animal use on a commercial scale.<sup>12</sup> As the clinical picture is beginning to unfold as a result of the years of increased usage and careful patient follow-up, it is conceivable that promising data may foster the development of DNA vaccines into one of the tools against human diseases in the public health arsenal.

### **Skin Patch Vaccine—Son Of The Gun: How Did One Step Lead To Another?**

I had a misconception when I started working on the gene gun; i.e., “vaccines have to be inoculated into a bodily compartment in order to trigger an immune response.” However, evidence showed that the magnitude of an immune response was inversely correlated with the depth of cutaneous penetration of DNA following bombardment with a gene gun.<sup>13</sup> As the outer layer of skin is in frequent contact with environmental pathogens, it is logical for the immune system to deploy the most competent “immunological soldiers” along the border to ward off infections. When vaccines are injected into muscle as commonly practiced,<sup>14</sup> the immunocompetent



#### **About Dr. Tang**

After studying Biology at Tunghai University (Taiwan), Dr. De-chu Christopher Tang moved to the United States for his PhD, which he completed in 1989 at Indiana University (Bloomington, IN). He performed postdoctoral studies at Baylor College of Medicine (Houston, TX) and Duke University (Durham, NC). During 1990–1994, Dr. Tang was affiliated with the University of Texas Southwestern Medical Center (Dallas, TX) as Assistant Instructor/Instructor. From 1994 to 2004, Dr. Tang was affiliated with the University of Alabama at Birmingham (AL) as Assistant/Associate Professor in the Departments of Medicine, Biochemistry and Molecular Genetics, and Dermatology and as Scientist at its Comprehensive Cancer Center and the Gene Therapy Center. Dr. Tang founded the company Vaxin Inc. in 1997, for which he served as board member (1997-1999), Vice President of Research and Chief Scientific Officer (1997-2012). Subsequently, he moved to Korea for a position as Korean Brain Pool Program Scientist at Chung-Ang University and the International Vaccine Institute, both located in Seoul (2012–2013). He is currently a Scientist at the International Vaccine Institute.

The development of noninvasive vaccines capable of conferring rapid-sustained-broad protection against infectious agents without the hazard to induce tissue-destructive systemic inflammation is one of Dr. Tang's major research interests. Dr. Tang and his associates were the first to have developed DNA vaccines, skin patch vaccines, adenovirus-vectored nasal influenza and anthrax vaccines, the protective innate-adaptive immunity duo platform technology, as well as adenovirus-vectored *in ovo* vaccines capable of mediating mass-immunization of poultry to mitigate future pandemics. He and his associates have demonstrated an excellent safety profile of non-replicating adenovirus-vectored nasal influenza vaccines by performing two human Phase I clinical trials.

His research has resulted in over 50 publications in peer-reviewed scientific journals, and more than a dozen patents related to innovative vaccines that have generated deep and broad trends with the promise to lift public health to a higher caliber. Dr. Tang is regularly invited to speak at national and international meetings, and he serves as Editorial Board member for several journals in the field of vaccines. He has received numerous honors and awards, such as the Year 2000 Wallace Coulter Award for Innovation and Entrepreneurship (Atlanta, GA), and the Distinguished Overseas Scientist Award in 2012 from the Korean Brain Pool Program (Seoul, Korea).

outer layer of skin that can be targeted easily is actually missed.

Since the deeper, the worse, why is a penetration device (e.g., gene gun; syringe needle) required for vaccine delivery? When I established my own laboratory at University of Alabama at Birmingham (UAB) in 1994, I challenged the contemporary method for vaccine delivery by pipetting a drop of an adenovirus serotype 5 (Ad5) vector encoding a human protein onto unbroken murine skin post-depilation and allowed incubation to continue for approximately 30 min until the animal

woke up from anesthesia. To everyone's surprise, serum antibodies to this human protein were elicited in mice a month later.<sup>15</sup> This demonstration represented the debut of noninvasive “skin patch vaccines”; it was also an epitome of how one step may lead to another. Owing to its obvious commercial potential, I received a number of inquiries with regard to investment. Three months after the paper<sup>15</sup> was published, Emerging Technology Partners (ETP) and I formed a covalent bond to launch the biotech company Vaxin Inc. on UAB campus with a focus on the

development of skin patch vaccines which represented a cutting-edge technology at that time. A few months later, others reported that mice could be immunized by topical application of adjuvanted proteins.<sup>16</sup> We subsequently demonstrated that topical application of  $\gamma$ -irradiated non-replicating whole *Escherichia coli* particles over-producing pathogen-derived antigens could immunize animals against live pathogens in a single-dose regimen.<sup>17</sup> Taken together, these findings provided compelling evidence that skin patch vaccines can comprise biomolecules that are too large for physical penetration into a bodily compartment through skin. Instead of absorbing vaccines into the body, the cutaneous immune system can selectively respond to large foreign antigens that are trapped on top of skin for eliciting an immune response.

Since the skin surface is loaded with a large number of commensal bacteria,<sup>18</sup> it is intriguing why topical application of a benign laboratory strain of *E. coli* would trigger such a potent immune response.<sup>17</sup> It is conceivable that the enteric *E. coli* bacterium is tolerated in the digestive tract but not on the skin. A  $\gamma$ -irradiated *E. coli* vector or other vectors (e.g., yeast vector) over-producing foreign antigens hence may be developed into semi-synthetic vaccine capsules capable of delivering a pathogen-derived antigen in its native configuration to the outer layer of skin adjuvanted by benign microbial components for activating the immune system, without the requirement to purify antigen proteins in vitro.

To date, human subjects have been effectively immunized by topical application of either adjuvanted protein<sup>19</sup> or Ad5-vectored vaccine.<sup>20</sup> The immunologic competence of the skin, the ease with which vaccines can be targeted without trauma to defined sites on the skin after ablating stratum corneum, the rapid turnover of skin cells, and the efficacy of skin patch vaccines collectively provide substantial justification for the development of a new class of vaccines that can be easily administered by non-medical personnel without inducing pain, fear, and systemic inflammation.<sup>14</sup>

As a potential game-changer, skin patch vaccines have been further developed by a

number of groups<sup>7,21</sup> and this class of non-invasive vaccines, conceivably, may cross the finish line toward licensure in the foreseeable future.

### **Innate-Adaptive Immunity Duo Activated by Noninvasive Vaccination: A Prototype of Future Vaccines**

When ETP and I launched Vaxin in 1997, my business partner told me to take time developing high-impact technologies with high commercial stakes. Their goal was to build a San Diego in Birmingham; not to make quick money. When the ETP Founder moved to Utah, the new Director changed ETP's nameplate as well as its vision. Through a myopic lens, biotech was a risky business and development of a new vaccine would take too long for investors to reap profits. As a consequence, the new Director recruited consecutively several CEOs into Vaxin with a mission to sell the company.

One of the CEOs came to Vaxin with a background on the development of a nasal influenza vaccine containing cold-adapted live-attenuated influenza virus (LAIV; known as FluMist® in the US). He decided to focus on the development of a non-replicating Ad5-vectored nasal influenza vaccine to outcompete FluMist® that was developed initially under his watch; hence Vaxin's core technology (i.e., the skin patch vaccine) was strategically moved to the back burner. Although the decision was a blow to the development of skin patch vaccines, I was animated by the opportunity to develop nasal vaccines which represent another class of noninvasive vaccines that can be painlessly administered by personnel with a low level of medical skill.

The choice of Ad5 as the vector system paved the way to both a financial desert and the land of opportunity. The Ad5 vector has been invasively administered into animals and humans by injection during a large number of trials with results showing that the rapid development of anti-Ad5 immunity following the first injection of Ad5 may impede its clinical use; moreover, pre-existing Ad5 immunity is commonly found in human populations.<sup>22</sup> Prejudice against Ad5 as a vector system

has made it difficult to raise capital investment in support of the development of an Ad5-vectored vaccine.

However, emerging evidence shows whether animals can be effectively immunized repeatedly by an Ad5-vectored vaccine hinges on the route of administration. It has been demonstrated that, in contrast to invasive injection, intranasal administration (the natural route of Ad5 infection) would allow an Ad5-vectored vaccine to bypass pre-existing Ad5 immunity without appreciably losing potency in mice,<sup>23,24</sup> nonhuman primates,<sup>25</sup> and humans;<sup>20</sup> conceivably attributed to the high efficiency of gene delivery, robust transgene expression, and potent antigen presentation along the mucosal barrier in the respiratory tract. Pre-existing Ad5 immunity is thus not a limiting factor if the Ad5 vector is bioengineered into a nasal vaccine carrier. We have demonstrated that intranasal administration of Ad5-vectored vaccines could elicit immune responses against pathogen-derived antigens with an excellent safety profile in both animals<sup>24,26</sup> and humans,<sup>20</sup> even in the presence of pre-existing Ad5 immunity. Misunderstanding of Ad5's potential and limitation has thus provided a rare opportunity for a small entity to develop Ad5-vectored nasal vaccines when big pharma companies either keep away from this vector or administer it invasively by intramuscular injection which appears to be a wrong way to do the right thing.<sup>14,22,27</sup>

In addition to eliciting protective immunity as contemporary vaccines do, we demonstrated serendipitously that intranasal instillation of the E1/E3-defective Ad5 particle, even without transgene, could confer nearly-immediate and broad protection of animals against lethal challenges with live pathogens,<sup>14,22,27,28</sup> conceivably due to activation of discriminating arms of innate immunity. Furthermore, Ad5-induced protective innate immunity did not decline away until vaccine-induced adaptive immunity was fully engaged.<sup>28</sup> These findings potentially may foster the development of a drug-vaccine duo (DVD) platform capable of conferring rapid-sustained-broad protection of vaccinees against infectious agents in one fell swoop. Revealing the



underlying mechanisms would expand the dimensions of our knowledge database for understanding the dynamic interactions between microbes and the host immune system beyond what contemporary textbooks teach.

This innovative DVD technology invited an unforeseeable problem: When a new CEO stepped into Vaxin, he made it clear that the development of DVD was not on his radar screen and it would never be. Since I was not in sync with this CEO's business model, I moved to South Korea by accepting the Korean Brain Pool Program Award in 2012 with a dedication to follow science, reason, and evidence wherever they lead. I subsequently joined International Vaccine Institute (IVI) in Seoul to continue my research on projects that may be developed into high-impact science in compliance with IVI's mission to "discover, develop, and deliver safe, effective, and affordable vaccines for developing nations." To my vision, future vaccines should confer rapid-sustained-broad protection of vaccinees against pathogens (beyond what contemporary vaccines can do in a slow motion); they have to be mass-produced at low costs and mass-administered painlessly by personnel without sophisticated medical training (vaccinate simple, so people can simply be vaccinated); in addition, injectable vaccines ought to fall by the wayside since they tend to induce systemic inflammation which is associated with the etiology of a wide range of diseases (both acute and chronic) whereas inflammation induced by noninvasive vaccines tends to be restricted to the periphery (where tissues are constantly replenished with new cells) without the hazard to simmer internal organs.<sup>14</sup> There is thus an enormous public benefit to unburden vaccinees by switching from injectable vaccine to noninvasive DVD that potentially can lift public health to a higher caliber by meeting these humane requirements. A challenge ahead is to demonstrate that nasal spray of non-replicating vectored vaccines can confer nearly-immediate as well as sustained protection of human subjects against live pathogens without inducing harmful systemic inflammation<sup>14</sup>—stay tuned.

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