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Review

Lessons from next generation influenza vaccines for inflammatory disease therapies

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ABSTRACT

Lessons can be learned for treating inflammatory diseases such as rheumatoid arthritis (RA) from next generation approaches for development of universal influenza vaccines. Immunomodulation of inflammatory diseases, rather than ablation of cytokine or cellular responses, can address the root cause of the disease and provide potential cure. Like influenza, there are different antigenic 'strains' and inflammatory T cell responses, Th1 or Th17, that drive each person's disease. As such, next generation vaccine-like antigen specific therapies for inflammatory diseases can be developed but will need to be customized to the patient depending upon the antigen and T cell response that is driving the disease.

1. Introduction

The next generation of vaccines will extend beyond antimicrobial targets to include antigen specific therapy for cancer and autoimmune and inflammatory diseases, such as rheumatoid arthritis (RA). Whereas vaccines prevent disease, these antigen specific therapies will provide treatment for ongoing diseases. Many of the lessons for developing these new therapies can be obtained from the approaches that are being used to solve the problems with prevention and treatment of influenza. Tissue damage, new antigen responses and excess inflammatory cytokine production during infection and inflammatory disease cause and exacerbate the subsequent disease [1,2].

Billions of dollars are spent on therapies to ameliorate but not cure or prevent the symptoms of influenza and RA in the form of cold remedies, NSAIDs, and for RA, ablative therapies to curtail the pathogenic inflammatory response. For influenza, prevention is still better than therapy and the best means of preventing influenza disease is vaccination. Unfortunately, paraphrasing the title for a recent article in the journal "Science", the flu vaccine suffers from mediocrity [3] (discussed below) with the hope that next generation vaccines that are in development will remedy the problems of the current vaccines. We propose that better prevention of influenza and treatment of RA requires attention to activating the appropriate T cell responses that promote protection and modulate rather than initiate unwanted inflammatory responses. Next generation vaccines must:

1. Demonstrate beneficial activity towards the disease
2. Block or modulate disease driving responses, whether microbial or immunological, to cure rather than just treat symptoms
3. Act through antigen specific responses
4. Allow assay for efficacy.
5. Drive appropriate modulation of immune response by up and/or down regulation of appropriate T cells and cytokines.

2. Influenza

Unlike measles, mumps, rubella and smallpox, for which vaccination is sufficient to elicit protection against all strains of the virus, influenza requires annual immunizations due to the numerous strains of virus and its propensity to create new strains through mutation or reassortment of the genomic strands of the human and animal strains of virus. The annual 'flu' shot is meant to provide this protection by eliciting protective antibodies to the prevalent strain of virus but the manufacture of these vaccines begins a year in advance of administration based on an epidemiologic prediction of the predominant annual influenza strains. In addition, most of the commercial vaccines are adapted to and produced in fertilized eggs which can select for avian traits and antigenicity. Only some of the vaccines remain as human viruses during production in human tissue culture cells or by genetic engineering (Table 1).

The annual vaccine is tested by evaluating antisera generated by test batches of the vaccine using a surrogate marker of efficacy,

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Table 1
Parameters of protection of representative current and next generation Influenza vaccines.

	Licensed current generation influenza vaccines (seasonal only)		Next generation universal influenza peptide vaccine candidates		
Agent(s) and company	Sanofi, GSK, ID Biomedical Seqirus, Aventis Medimmune	Protein Sciences	M-001 by BiondVax [44]	FLU-v by SEEK & hVIVO [10,45]	LEAPS Uni-Flu by Cel-Sci [16]
Form of vaccine	Inactivated, purified multiple antigens, or attenuated virus	Recombinant HA protein	Fusion protein of several epitopes from different proteins and flagillin for adjuvanicity	Peptides	DCs matured with J-NP & J-M2e peptides
Protection in animal studies	[46–49]	[50]	[13–15]	[10,11,45]	[16]*
Antibodies	Hemagglutination inhibition (HAI)	Hemagglutination inhibition (HAI)	TBD for HAI	TBD (specificity and isotype in mouse)	Anti-NP, M2e & total Abs: IgG2A (Th1 marker) > IgG1 (Th2 marker)
IL1β	ND	ND	ND	ND	↓ ILβ1
IL2	ND	ND	TBD in phase II trial	TBD in phase II b trial	↑ IL2
IL4	ND	ND	Not done	TBD in phase II b trial	↓ IL4
IL6	See [46] also for IL10	ND	ND	ND	↓ IL4
IL12p70	ND	ND	ND	ND	↑ IL12p70
IFNγ	See [46]	[50]	TBD in phase II trial	↑ IFNγ (in phase 1b trial) TBD in phase II b trial	↑ IFNγ
TNFα	ND	ND	TBD in phase II trial	TBD in phase II b trial	↓ TNFa
CD107a (CTL)	ND	ND	TBD in phase II trial	TBD in phase II b trial	ND
CD4 T	ND	ND	ND	TBD in phase II b trial	↑ CD4+ cells in infected lungs & NP tetramer response
CD8 T and or Granzyme B	See [47]	ND	ND	TBD in phase II b trial	ND
Perforin	ND	ND	TBD in phase II trial	ND	ND
DC	ND	ND	ND	ND	↑ LEAPS activated DC in infected lungs

* Tested in model with ongoing influenza infection which may have different cytokine levels compared to no infection [16].

hemagglutinin inhibition (HI), as per recommendations of the FDA's Center for Biologics Evaluation and Research (CBER) [4,5], rather than protection from infection. HI tests the ability of antibodies to block the binding of the virion glycoprotein (hemagglutinin (HA)) to erythrocytes. Evaluation of next-generation of influenza vaccines is likely to require other means of evaluation since HI is not suitable to evaluate other immunogens (e.g. neuraminidase (NA)) or the positive and negative aspects of newer vaccines that incorporate adjuvants (e.g. Fluad) and elicit T cell responses [6].

The challenge for the next generation of influenza vaccines is to alleviate the need for annual immunizations with a universal vaccine that will elicit protections against most influenza A strains and possibly an immunotherapy that will rapidly elicit protection from disease even after exposure to the virus [7]. A promising approach focuses the immune response on less variable, shared regions of the HA molecule. Even this approach may be frustrated since annual immunization reinforces and focuses the antibody response generated towards the earliest and antigenically most dominant viral HA experienced by the individual at the expense of more recent and potentially more pathogenic strains of the virus, a phenomenon called original antigenic sin [8].

As an alternative to antibody protections to influenza, T cell responses target the infected cell rather than the free virus, are not as strain dependent, can prevent influenza disease, should provide broader coverage against the annual virus and can be more long lasting [9]. The live attenuated influenza vaccine (LAIV) and adjuvanted influenza vaccines, such as Fluad, elicit T cell responses but these vaccines still require annual immunizations because the primary protection is elicited by strain specific antibody. Taking lessons from “Mother Nature”, the antigens included in a T cell inducing influenza vaccine would be the ones that normally elicit strong T cell responses during infection. These would include the matrix (M1), membrane (M2e) and nucleoprotein (NP) viral proteins that are present within the infected cell. The vaccine may take on any of the following forms, protein, peptide, DNA or RNA, hybrid virus vaccine or adoptive transfer of antigen stimulated DCs or T cells [9]. Peptide vaccines offer the opportunity to deliver a mixture of antigenic determinants capable of eliciting defined responses in individuals with different MHC specificities.

In addition to protecting against influenza disease, T cell responses are also responsible for eliciting some of the symptoms of an influenza infection by activating inflammatory responses [1]. These inflammatory responses are driven by Th17 and Th1 T cell responses. Th17 responses are induced by TGFβ plus IL6 or IL23 and characterized by the production of interleukin 17 (IL17), IL22 and TNFα and promote neutrophil and other inflammatory reactions while Th1 responses are induced by IL12 and characterized by the production of interferon γ which activates cytolytic CD8 T cells and macrophages to promote antimicrobial and inflammatory responses. The challenge for a next generation influenza T cell vaccine would be to present the T cell antigenic proteins or their parts (peptide epitopes) in a manner that elicits an optimal T cell protective but not inflammatory response to elicit protections against many strains of the virus in the broadest population.

The Flu-v (sponsored by Seek Group) [10,11] and multi-epitope fusion protein Multimeric-001 (M-001) (NCT03058692 sponsored by BiondVax) [12–15] vaccines are two examples of T cell immunogens developed as universal influenza vaccines. Both of these vaccines are undergoing clinical phase II b studies. Since antibodies are often not elicited by T cell vaccines, the vaccines were evaluated by alternative surrogate markers of efficacy including cytokine production, such as IFNγ, IL12p70; T cell markers indicative of activation, such as CD107a (for activated T and NK cell activity); production of the T cell effector molecules perforin and Granzyme B (for CTL activity); in addition to protection in animal models.

Combining the peptide approach with adoptive transfer of dendritic cells (DC), Boonnak et al. [16] evaluated a therapeutic vaccine consisting of DCs matured in the presence of a heteroconjugate J-LEAPS peptide. The J-LEAPS-DCs elicited T cell protection in mice as late as 2 days after initiation of disease and modulated inflammatory responses in a timeframe that was too short to elicit antibody. The LEAPS peptide vaccines consist of an immune cell binding ligand (ICBL) attached to an antigenic peptide containing at least one T cell epitope. The ICBL promotes and directs the nature of the immune response to the peptide. The J-ICBL elicits Th1 responses [17–19] whereas the G or DerG-ICBLs elicits Th2 and Treg responses [17,20,21]. J-LEAPS vaccines have been tested in other mouse models and elicit protective responses to lethal challenge with HSV-1 [22–24] and prevention and therapy for murine HER-2neu positive tumors [25], encephalomyocarditis and RA [26,27] by eliciting IFNγ driven T cell responses. The J-LEAPS vaccines are sufficient to activate and promote the maturation of mouse and human precursors to become DCs that promote Th1 responses [28]. When matured with a J-LEAPS vaccine incorporating a peptide from the gD protein of HSV-1, adoptive transfer of the resultant JgD-LEAPS-DCs was sufficient to elicit protection from lethal challenge with HSV-1 [29].

The protective anti-influenza J-LEAPS vaccines incorporated CD8 T cell epitopes containing peptides from the M2e or NP proteins [16]. J-LEAPS vaccine conjugates that incorporated CD4 T cell or antibody inducing epitopes from HA were not effective. The mice were analyzed for survival and symptomatology, daily weight changes, and on day 5 post administration, the presence of virus and antigen specific CD4 and CD8 T cells in lung tissues and 12 different cytokines in lung homogenates. In contrast to the mice protected by JM2e-LEAPS-DC or JNP-LEAPS-DC which had reduced levels of IL1b, TNFα, IL4 and IL6, the control and mice immunized with JHA-LEAPS-DCs exhibited inflammatory pathogenesis characterized by cytokines consistent with a human cytokine storm. This vaccine approach would require personalized vaccine development but would allow a rapid treatment response after exposure to a broader spectrum of influenza A viruses.

Licensure of T cell eliciting vaccines for influenza will require significant changes in vaccine philosophy and the parameters for determining efficacy. Alternates to HI must be developed as surrogates of protection and to determine appropriate dosing. The task may become easier since the broader antigenic efficacy of these vaccines could obviate the need for the development and annual testing of influenza vaccines.

3. Rheumatoid arthritis

RA, T1D, psoriasis and other inflammatory diseases are driven by dysregulated cell mediated immune responses. Th1 or Th17 responses are the primary drivers with antibody and the B cells that are making antibody contributing to the disease process. B cells can play a regulatory role and modulate inflammation [30,31] but they are excellent antigen presenting cells that present a focused repertoire of peptides to activate T cells and promote the inflammatory response [32]. Inflammatory responses can be monitored by the presence of acute phase (TNFα, IL1, IL6), Th1 (IL12, IFNγ, IL2, and TNFβ) and Th17 (IL23, IL17 and IL22) related cytokines in serum or an unbalanced ratio of Th1 and Th17 to Treg or Th2 cells and their respective cytokines, TGFβ and IL10 and IL4 and IL10 [33].

Current therapy for autoimmune and inflammatory disease is targeted at limiting or alleviating inflammation or symptoms rather than addressing the cause of the pathogenic immune response. For RA and psoriasis, treatment includes inhibition or ablation of immune cells with methotrexate, steroids or DMARDS (disease-modifying anti-rheumatic drugs) that block inflammatory cytokine functions with kinase inhibitors, monoclonal antibodies or soluble receptors (e.g. etanercept, sekukinumab) (Table 2).

The development of next generation antigen specific therapy for autoimmune and inflammatory diseases will build on improved understanding of the antigen specificities and immune responses that are driving the pathogenesis [34]. The next generation of RA therapies should progress away from inhibition and ablation towards modulation therapy to increase regulatory and decrease inflammatory responses

Table 2
Comparison of representative RA biologic drugs and therapeutic vaccines.

	Current generation						Next generation			
Product/company name	Adalimumab/ Abvee; Etanercept/ Pfizer, etc.	Anakinra/Amgen	Tocilizumab/ Roche	Rituximab/ Biogen, Genetech	Abatacept/BMS	Tofacitinib/Pfizer; Baricitinib/Eli Lilly	CEL-4000/CEL-SCI	Dendright/Jansen a)	Dendright/Jansen b)	CEL-2000/CEL-SCI
Information, other & source	Manufacturer's information	Manufacturer's information	Manufacturer's information	Manufacturer's information	Manufacturer's information	Manufacturer's information	[21]	[41]	ANZCTR phase I clinical trial # ACTRN 12617001482358	[27]
Molecule(s) delivered, type & route	Mab or sTNFαR; IV	Mab; IV	Mab; IV	Mab; IV	Mab; IV	Jak inhibitor, small drug; oral	Peptide; IM	a) Permanently arrested autologous cells & peptide; SC	b) Peptide SC	Peptide; IM
Target & action down (↓)	↓ TNFα	↓ IL1	↓ IL6	↓ CD20+ B cells	↓ CD80+ T cells	↓ IL17	Acts on Th1 driven inflammation ↓ IL1,-17, IFNγ, TNFα	↓ IL-6 by T cells; ↓ IL-15,-19 serum	↓ IL-6 by T cells; ↓ IL-15,-19 serum	Acts on Th17 driven inflammation ↓ IL-6, -17, TNFα
Target & action up (↑)	NR	NR	NR	NR	NR	NR	↑ Treg (FOXP3+), IL-4, -10, TGFβ	TBD	TBD	↑ IFNγ, IL-10, -12

Conclusions

1. Current RA conventional drugs target only one activity for either one cell type or cytokine or enzyme; only act in one direction down (↓), have systemic (total body) effect, delivery disadvantages for many, and counterindicated for many conditions
2. Whereas therapeutic (including LEAPS) RA vaccines have effect on multiple different types of cells and/or cytokines and influence either up (↑) and down (↓) on different targets only involved antigen specific cells or their secreted cytokines close to that cell's location

(see Table 2). Unlike antiviral vaccines, the antigen specific therapy for RA will incorporate a therapeutic immunogen that represents a self-antigen recognized by T cells (e.g. collagen or proteoglycan) rather than a foreign antigen that elicits antibody. Activation of regulatory responses can be obtained by various means as reviewed in Rosenthal et al. [34] Antigen specific therapy will depend upon progress that has been made towards identifying the responsible antigens, the immunological disease process, the potential for protective responses and the immunological means to regulate these processes.

An antigen specific therapy should focus on the relevant auto-antigen(s) and the disease driving inflammatory T cell response rather than ablate responses that are important for certain immune protections. For RA, T1D and MS, some disease related T cell antigens have been identified [34]. The challenge is to activate therapeutic rather than inflammatory responses to these antigens. Special approaches are required to activate the beneficial regulating/modulating T cells (Treg or TR1 cells) and other T cells (including humoral response promoting Th2 cells) that can provide treatment. An additional problem for antigen specific therapy is that different approaches may be required for modulating the disease driving T cell response since the inflammatory response driving disease, whether Th1 or Th17, may be different in different individuals (as demonstrated in animal studies) [34].

Upon reviewing the literature, there are relatively few examples of immunomodulating but not ablating vaccine therapies developed for RA. Potential approaches for antigen specific therapy for RA include co-administration of antigen and an immunosuppressive treatment, such as IL4, IL10, or a DNA plasmid encoding these cytokines [35]. Other suggestions are administration of apoptotic cells that expose tolerizing structures with covalently affixed peptide or protein autoantigens [36,37], or use of biodegradable poly (lactic-coglycolic acid) nanoparticles [38]. De Groot et al. have proposed use of immunomodulatory peptides, such as peptides within immunoglobulin called Tregitopes [39], linked to the antigen peptide to activate the appropriate response. A DNA vaccine that expresses chicken type II collagen was therapeutic and induced increased serum levels of regulatory cytokines in a collagen induced rat model of RA [40]. Dendright has experimented with other means for modulating inflammatory responses. They used NFκB inhibitors and autologous cells with a proteoglycan (PG) peptide antigen to promote a dendritic cell-like therapy [41]. More recently, their DEN-181 vaccine is a nanoparticle-based immunotherapy containing calcitriol and collagen II peptide antigens (peptide 259–273 (Proline 273 Hydroxyproline substitution)) designed to regulate activated immune cells and is in phase 1b studies [42].

Taking a lesson from the J-LEAPS DC influenza vaccines [16], therapeutic LEAPS peptide antigen specific therapies for inflammatory diseases were developed to specifically modulate Th17 or Th1 inflammatory responses [26,27]. CEL-2000, a J-LEAPS vaccine similar to those that provide protection against HSV-1 [22–24] and therapy for HER-2 antigen bearing breast cancer tumors [25], promotes IFNγ responses that modulate the Th17 inflammatory response [26,27]. In contrast, CEL-4000, a derG-LEAPS vaccines, provides antigen specific cessation of Th1 driven disease progression [21].

For RA, the efficacy of CEL-2000 and CEL-4000 LEAPS vaccines was demonstrated by therapeutic cessation of disease progression in their respective mouse models and modulation of T cells and serum cytokines away from inflammation and towards a healthier balance. CEL-2000 consists of a J-LEAPS vaccine incorporating a peptide from collagen type II [27] whereas CEL-4000 consists of a derG-LEAPS vaccine incorporating a peptide from proteoglycan (PG) [21]. CEL2000 administration to mice with ongoing RA disease in the CIA model reduced levels of Th17 cytokines (IL17, TNFα, IL1 and IL23) that drive disease by increasing levels of the immunomodulating IL10, IL12 and IFNγ cytokines. CEL4000 reduced levels of Th1 and Th17 cytokines by increasing levels of IL4, IL10, and TGFβ cytokines and FOXP3 expressing Tregs in the GIA model of disease in which Th1 responses drive disease. To date, there does not appear to be any untoward effects of the CEL-2000, CEL-4000 or other LEAPS antigen specific therapies.

4. Conclusion

The advantages of an immunomodulating Next Generation antigen specific therapy approach rather than ablative approach to treating RA are presented in Table 2. Current therapeutic approaches for rheumatoid arthritis block, inhibit or kill the pathways, cytokines or cells that promote inflammatory processes with small molecule inhibitors, monoclonal antibodies or soluble hybrid receptors. The ablation of these important processes does not provide a universal therapy for everyone but does increase risk to infection and other side effects. In contrast, the experimental antigen specific therapies described above act by selectively increasing (up arrow) modulatory/regulatory cytokines and cells while decreasing (down arrow) Th1 (IFNγ) or Th17 (IL17, IL22, TNFα, IL1 or IL23) cytokines and cells. As with all treatments, these antigen specific therapies must be proven safe and demonstrate that they do not elicit or exacerbate autoimmune responses in the recipient. The potential of antigen specific therapies for RA will become more evident as they progress beyond animal studies and through human trials.

We must acknowledge that next generation antigen specific therapies for RA will need to be customized for the antigen and immune response, Th1 or Th17, causing disease in each patient. This will require new, improved and standardized approaches for assessing the efficacy of RA antigen specific therapies for each individual. In the long term, success may be monitored by reduction in anti-CCP or other disease related antibody levels. A more timely test would assay T cell function by evaluating specific cytokine responses of an individual patient to a vaccine therapy in a manner similar to the IFNγ release assays for *Mycobacteria tuberculosis* [43]. For these tests, patient blood cells are challenged with *M. tuberculosis* antigen and IFNγ production is assayed. Increased levels of specific serum cytokines (IL10, TGFβ) with reduction in the levels of inflammatory cytokines (IL1, IL6, TNFα, IL12, IL23, IL17, IL22, INFγ) upon challenge with teh appropriate RA antigen in this assay would provide a relatively rapid indication of efficacy.

5. Summary

T cell antigen specific therapy represents the Next Generation for immunoprotection and immunotherapy. It provides the means for therapy by increasing the appropriate and decreasing the immunopathogenic responses rather than generic ablation of potentially helpful cytokine and cellular responses. For influenza A, a T cell vaccine would offer a more universal, less strain dependent protection. For therapy for RA and other autoimmune diseases, antigen specific T cell immunomodulatory therapies would provide the opportunity for personalized medicine that treats the cause of disease rather than just the symptoms.

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Work effort

DHZ conceived of the idea and concept behind this paper, as well as prepared the title, first draft, initial organization, tables, collaborated with KSR on editing, reorganization of subsequent versions, literature

search and selection.

KSR worked on concept, content, organization, writing, wordsmithing, collaborated with DHZ on editing of subsequent versions, literature search and selection.

RC worked on bibliography, reference manager and editing with others.

JC worked on literature search and selection for specific areas and editing with others.

Declaration of Competing Interest

DHZ, RC and JC are employees of CEL-SCI, stockholders and inventors of one or more LEAPS patents, DHZ is as well an officer of CEL-SCI. KSR is an inventor of one or more LEAPS patents and has no other conflict.

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