Current Studies of Therapeutic Vaccines for Rheumatoid Arthritis: Mechanism and Efficiency in Clinical Trials

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Abstract. Rheumatoid Arthritis is an autoimmune disease with systemic inflammation chronically progressing, resulting in functional impairment, disability, and premature mortality. While treatments for RA have developed rapidly in recent decades and more types of drugs have been made, current mainstream treatments only focus on easing the symptoms, inhibiting inflammation, or delaying disease progression without antigen specificity. Therapeutic vaccines for RA, however, come as a new promising RA therapy, as they focus on the upstream of disease presentation, which is antigen-specific disease-driving cells, to modulate antigen-specific autoimmune responses without hurting those against foreign antigens. This article overviews a range of RA therapeutic vaccines under current development, introducing their basic principle, experiment design, results, and limitations. It also provides prospects for RA therapeutic vaccines.

Keywords: Rheumatoid Arthritis; therapeutic vaccine; Rheumavax; DEN-181; CEL-4000.

1. Introduction

Rheumatoid Arthritis (RA) is an autoimmune disease with systemic inflammation chronically progressing, resulting in functional impairment, disability, and premature mortality [1-5]. Within joints, the characterized pathology of RA includes severe pain, as well as damage of articular cartilage and subchondral bone, which serve significant roles in maintaining the normal function of the capsule, ligaments, and tendons [2, 4]. Beyond articulation, RA also affects a wide range of organs such as the heart, lung, kidney, eye, and nervous system [4].

Though the prevalence of the disease has been greatly increasing in different countries since 1990, it is now small and is spread from south to north [4]. Nowadays, with a 0.5-1% prevalence globally, RA leads to a standardized mortality ratio of 1.28–2.98, among which more than 50% of premature deaths are directly caused by cardiovascular diseases [6]. The wide spectrum of manifestations of RA burdens the individual, the family, and the whole society, especially in aspects such as societal health, social security provision, healthcare resources, and loss of productivity and social economy [6].

Research on RA has gained important results in recent decades and proposed a variety of bio-molecular mechanisms. Nonetheless, the etiology of RA remains not yet fully understood. The currently known pathogenesis of RA can be concluded into a three-stage long-term progress consisting of the pre-RA phase, inflammation, and thus symptoms in turn. In the pre-RA phase, which can be years before manifest symptoms, due to the interactions between epigenetic modifications and environmental factors, a range of proteins, such as immunoglobulin G (IgG), are modified into self-antigens, which later on go through citrullination and thus become unrecognizable for the immune system [4]. Afterwards, these self-antigens initiate an immune response, generating varieties of inflammatory cytokines and promoting inflammation, bone erosion, and cartilage degradation, which eventually result in RA symptoms like pain [4]. Aside from the basic pathogenesis above, several complementary factors, including environmental pollutants, gut microbiota, angiogenesis, and signaling molecules, have also been found related with RA pathogenesis [4].

A range of approaches have been developed and tested in clinical trials to tackle the disease. The current mainstream treatments can be subdivided into four categories: non-pharmacological treatment, non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs), and disease-modifying anti-rheumatic drugs (DMARDs).

In RA, the disease manifestation and overall condition of patients can be seriously affected by pain, depression, and anxiety. Therefore, non-pharmacological treatments, in some cases surgery included, are employed to reduce pain and stiffness or relieve depression and anxiety. Among this category, polyunsaturated fatty acids (PUFAs), which have been shown to improve depression and thus control the symptoms, are commonly used [4]. Moreover, occupational therapy and physical exercises are also recommended in the clinic. Last but not least, surgery is sometimes performed in RA, though rates of which have shown low effective results in patients younger than 60 [4].

NSAIDs, such as aspirin, coxibs, diclofenac, ibuprofen, and naproxen, are drugs with no disease-modifying function but reduce systems of RA, particularly pain and swelling. This category of drugs performs their function mainly by inhibiting prostanoid biosynthesis, which promotes inflammation [7]. Effective as they are in controlling symptoms, NSAIDs are rarely used unless in the acute phase response because of their side effects, which include gastrointestinal ulceration, bleeding, heart failure, seizures, etc [4].

GCs are non-specific immunosuppressive drugs orally, intravenously, intramuscularly, or intra-articularly taken, which score higher than NSAIDs in both potency and side effects as a result of their complicated pharmacological mechanism [4, 7]. Despite the efficacy in delaying chronic progression GCs have shown, GCs are only used as either bridging therapy for DMARDs before their efficacy manifests, or adjunctive therapy in cases of severe symptoms even after using DMARDs [4]. If used in the long term as a major treatment, GCs give rise to a range of serious side effects such as ulcer formation, osteoporosis, muscle weakness, diabetes, and water retention [7].

DMARDs are a group of disease-modifying agents able to slow down RA progression and prevent further joint damage by suppressing autoimmune activity [4, 7]. The wide range of developed DMARDs can be grouped into biological DMARDs (produced by living cells and function on cytokines) and synthetic DMARDs (further subdivided into conventional and targeted synthetic DMARDs). The three types of DMARDs, biologic, conventional synthetic, and targeted synthetic, each have their beneficial effects and side effects.

Conventional synthetic DMARDs, with a rather low cost in comparison with the other two types as its advantage, have a well-characterized profile of side effects that includes rash, cytopenia, poor tolerability, and in some cases interstitial lung disease, and liver damage [7]. The mechanism of conventional synthetic DMARDs, however, remains not yet understood [7].

Biological DMARDs and targeted synthetic DMARDs, developed later than conventional synthetic DMARDs, are now on an upward trend, especially for the patients to whom conventional synthetic DMARDs are ineffective or poorly tolerated [4]. These two types of DMARDs are both designed based on key steps of cytokine-mediated induction of inflammatory responses and have shown efficacy in preventing joint damage [7]. They are, however, accompanied by higher costs, which limit their spread at present, as well as a range of side effects such as congestive heart failure, transaminases, induction or reactivation of multiple sclerosis and psoriasis, etc.

As aforementioned, current mainstream treatments focus on either simply easing the symptoms or delaying disease progression with drugs of which pharmacological mechanisms remain unclear [8]. This lack of disease- or antigen-specificity lays hazards for patients, with the probability of eliminating important immune responses and increasing infection rate, and thus limits current treatments [3, 8]. Additionally, the current management of RA demands early diagnosis, continuous pharmacological or nonpharmacological treatments, along with regular clinical check-up evaluation, which can all be hard for some of the patients [4]. In comparison, however, therapeutic vaccines stand out with their focus on antigen-specific disease-driving cells [8]. RA therapeutic vaccines cast new light on the cure of RA in a promising manner, as they can modulate upstream of RA pathogenesis with high specificity, regulating pro-inflammatory T helper cells (Th) responses or enhancing T regulatory cells (Treg) responses [8].

Therefore, it is of great importance to design a successful RA therapeutic vaccine, which shall solve the problems of current mainstream treatments listed above, and at the same time guarantee

efficacy and safety. This article aims to summarize updated research and trials on RA therapeutic vaccines, to help navigate future vaccine design and research.

2. RA therapeutic vaccines in development

2.1. Rheumavax

Dendritic cells (DCs), a type of antigen-presenting cells (APCs), are responsible for presenting antigens to T cells in draining lymph nodes. Trials on animals have found that DCs previously exposed to autoantigen (autoantigen-exposed immunoregulatory DCs) can suppress inflammatory arthritis with antigen-specificity [9]. Based on that, a therapeutic vaccine with autoantigen-exposed immunoregulatory DCs as its main component, designated "Rheumavax", was designed and went through its phase I trial.

In the trial, the study investigators modified DCs with Bay11-7082, which can irreversibly inhibit nuclear factor kB (NF-kB) and suppress antigen-specific immune response [3]. They then exposed the modified DCs to citrullinated peptides taken from RA patients that carry Anti-citrullinated protein antibodies (ACPA+), as well as human leukocyte antigen-shared epitope (HLA-SE) alleles [3]. A single subcutaneous dose of the modified, autoantigen-exposed DCs was then given to patients in the upper thigh (the flowchart of the trial is shown in Fig. 1) [3].

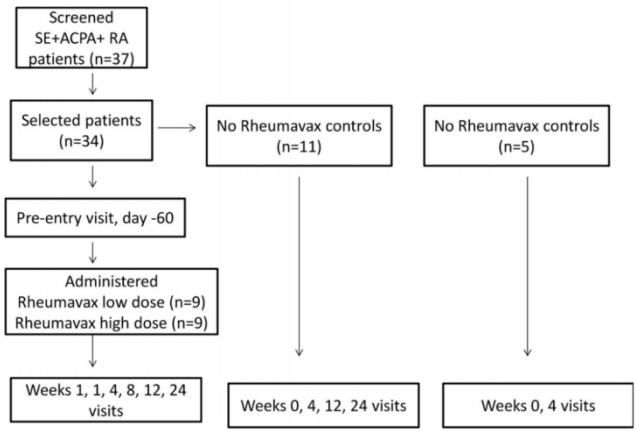

Fig 1. Flowchart of the Rheumavax trial [3]

The trial included a total of 34 ACPA+ RA patients, who were divided into a low dose Rheumavax treated group of 9, a high dose Rheumavax treated group of 9, and an untreated control group of 16 [2]. The treated groups have baseline characteristics with no significant difference from those of control group [3]. Changes in a series of indexes, including %CD4+ CD25+ CD127+ Teff and CD4+ CD25hiCD127−Treg, were assessed 6 days and 1 month after the injection, which is the usual time when DCs manifesting their effects on peripheral T cell immunity is expected [3].

As the results of the trial in Fig.2 show, a decrease in %Teff by ≥25% was observed within the first month after Rheumavax injection in 11 of 15 assessed patients [3]. As a contrast, a decrease in %Teff by ≥25% was seen in 0 of 5 patients in the control group [3]. As for the %Treg, an increase of≥25% was observed in 0 of 5 controls and only 5 of 15 treated patients [3]. The Treg/Teff ratio increase by ≥25%, however, it was observed in 1 of 5 controls and 11 of 15 treated patients, which is consistent with the shifted balance from regulatory into effector CD4+ T cells [3]. Aside from T cells, levels of IL-15, IL-29, CXCL3, and CXCL11, which were important pro-inflammatory cytokines involved in inflammatory arthritis, were also measured and proved an overall decrease in treated groups than controls [9]. Though varied between individual, IL-6 responses to vimentin447–455–Cit450 showed a decrease in vaccinated groups in comparison with controls [9]. Adverse events of Rheumavax, such as transient anemia, all scored 1 out of 4 for severity and were parallel in groups of different doses [3].

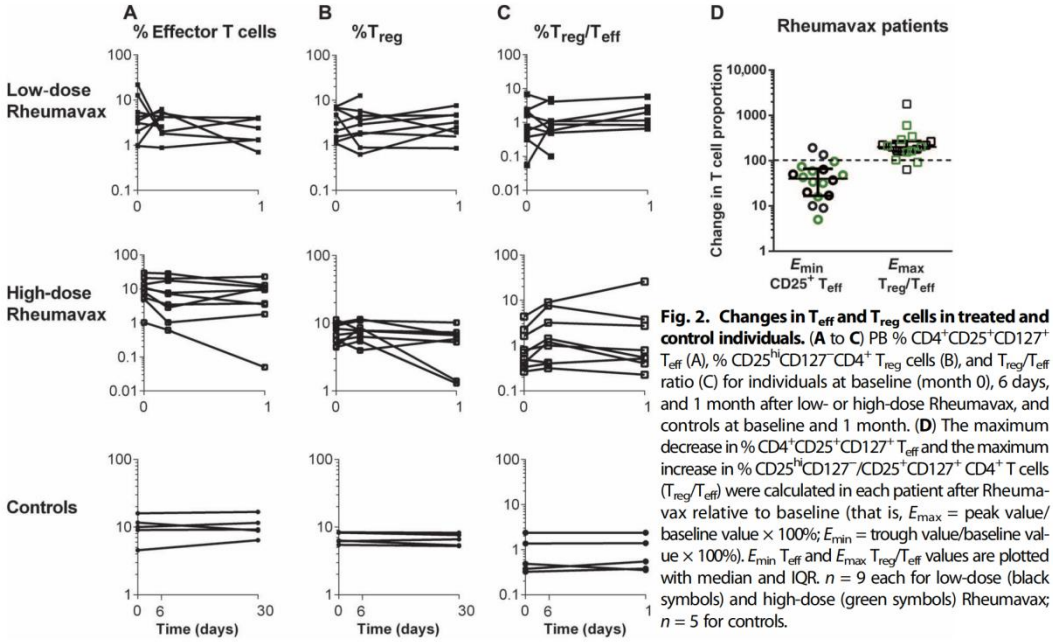

Fig. 2. Changes in T_{eff} and T_{reg} cells in treated and control individuals. (**A** to **C**) PB % $CD4^+CD25^+CD127^+$ T_{eff} (A), % $CD25^{hi}CD127^-CD4^+$ T_{reg} cells (B), and T_{reg}/T_{eff} ratio (C) for individuals at baseline (month 0), 6 days, and 1 month after low- or high-dose Rheumavax, and controls at baseline and 1 month. (**D**) The maximum decrease in % $CD4^+CD25^+CD127^+$ T_{eff} and the maximum increase in % $CD25^{hi}CD127^-/CD25^+CD127^+$ $CD4^+$ T cells (T_{reg}/T_{eff}) were calculated in each patient after Rheumavax relative to baseline (that is, E_{max} = peak value/baseline value × 100%; E_{min} = trough value/baseline value × 100%). E_{min} T_{eff} and E_{max} T_{reg}/T_{eff} values are plotted with median and IQR. n = 9 each for low-dose (black symbols) and high-dose (green symbols) Rheumavax; n = 5 for controls.

Fig 2. Changes in Teff and Treg in treated and control individuals [3]

The results of the trial on Rheumavax prove it quite promising as a new attempt to cure the disease. It modulates the autoimmune response antigen-specifically but does not suppress the immune response to foreign antigens like a lot of current treatments do [9]. Further future research is still needed, however, to confirm the results with more sensitive assays and larger sample sizes, figure out the best vehicles to modify the DCs, as well as investigate if individualized therapy improves its performance [3, 9].

2.2. DEN-181

Quite like Rheumavax, the vaccine "DEN-181" is also based on Antigen-Specific Immunological Tolerance (ASIT) strategies, achieved by leveraging the antigen-presenting and regulating functions of DCs [10]. Both vaccines, aimed at regulating the RA pathology and modulating autoimmune responses in an antigen-specific manner, modified Autologous DCs with NF-κB inhibitory drugs, after which the DCs were exposed to citrullinated peptides [10]. Unlike Rheumavax, however, DEN-181 is a vaccine with liposome encapsulating the RA joint-specific self-peptide collagen II259-273 (CII), as well as the NF-κB inhibitor 1,25 dihydroxycholecalciferol (calcitriol) [10]. The formulation has received promising results in regulating memory T (Tmem) cells, induced bystander tolerance, and reduced disease severity in animal trials [10].

In this placebo-controlled, double-blind, phase I clinical trial, a single s.c. site, 2 sites, or 3 sites of the vaccine DEN-181 were administered to patients divided into 3 cohorts [10]. The 17 patients have gone through prescreening for methotrexate (MTX) therapy, HLA-DRB1*04:01 or *01:01, and trail eligibility [10]. (The experiment flow is shown in Fig.3.) To evaluate the efficacy of DEN-181, a variety of methods, including cluster analysis and flow cytometric assay were used to test the Cit-Vim–specific T cell level, the phenotype of T cells, RA disease activity scores, etc. [10].

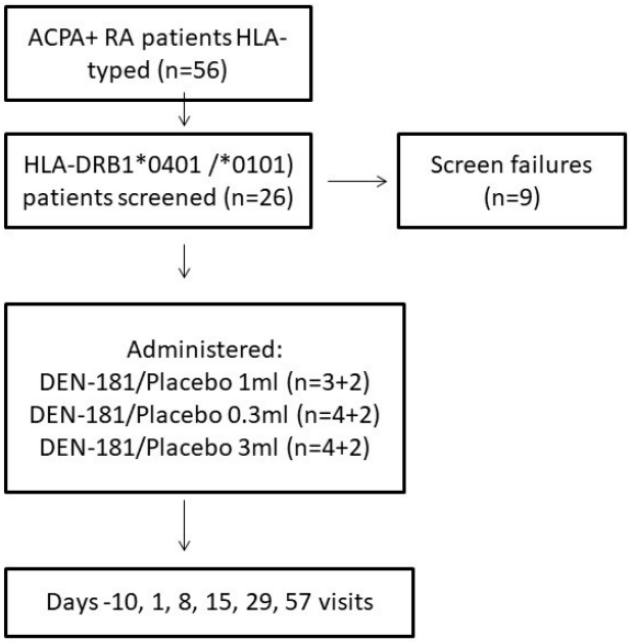

Fig 3. flowchart of the DEN-181 trial [10]

A significant increase in PD-1 expression, Tem cells, and Tcm cells among Cit-Vim–specific T cells has been observed compared to that of total CD4+ T cells, despite individual variability in the proportion of various kinds of T cells [10]. The CII-specific T cells of any dose vaccinated group also manifested more active state than that of a placebo, with higher expression of PD-1, HLA-DR, CD25/CD127, or Tfh markers [10]. Besides, the study investigators also noticed a decreasing trend of ACPA VDG in patients treated with 0.3 mL DEN-181, as well as an increasing trend in the 3 mL DEN-181 treated group [10]. No significant difference between placebo and treated groups, however, was observed in cell numbers, Fc ACPA percentage fucosylation, CD4+ T cell numbers, etc. [10]. As for potentially related adverse events, there were cases of elevation of alanine transaminase (ALT) or aspartate transaminase (AST), grade 2 joint synovitis, and injection site bruising [10].

While the study investigators concluded low/medium DEN-181 doses as a safe and antigen-specific therapy for ACPA+ RA patients, the trials do have such limitations as limited samples, MTX dose instability, and lack of multi-dose trials [10]. To further confirm the hypothesis of ASIT expanding and differentiating Tregs to suppress RA, trials with multi-dosing DEN-181 should be conducted in the future [10].

2.3. CEL-4000

Designated “CEL-4000”, the immunodominant proteoglycan (PG) peptide PG70 is attached to a DerG immune cell binding peptide to make this Ligand Epitope Antigen Presentation System (LEAPS) vaccine [8]. The LEAPS, consisting of a specific antigen epitope and an immune cell binding ligand (ICBL), enables antigen-specific immunization in certain diseases and immune response modulation in subsequence [8]. As one of the potential therapies for RA, the efficacy of “CEL-4000” have been tested in animal trials.

The trial was conducted on mouse models with Proteoglycan (PG)-induced arthritis (PGIA) and G1 domain (of PG) induced arthritis (GIA), both of which have been proved to well resemble RA in human [8]. The study investigators induced RA with intraperitoneal (i.p.) immunization of mice with human articular cartilage PG in anesthetized mice [8]. After immunizing twice with PG, they then observe signs of RA twice every week, and rate VA score every other day [8]. After that, the mice were sorted into treatment groups accordingly, with each group scoring similarly in average Visual Arthritis (VA), to receive vaccine treatments upon the onset of RA [8]. In this trial, efficacy of the

vaccine was evaluated through a range of outcomes including serum cytokine level, in vitro cytokine secretion, T-cell phenotypes, etc. [8].

As shown in Fig.4, the VA score of CEL-4000 treated group was significantly ($p < 0.05$) lower than that of control group within 3 weeks, which indicates its efficacy in limiting RA progression [8]. Moreover, the trial has also found the vaccine showed great performance limiting RA progression in GIA, which is harder than controlling that in PGIA [8]. As for histopathological changes, the vaccine significantly reduced inflammation, cartilage damage, and bone resorption as compared with controls [8]. The lower cumulative histopathology scores of vaccinated group further support CEL-4000's reproducible efficacy in reducing the pathology as well [8]. Other assays and flow cytometry to test serum and spleenocytes-secreted cytokines in vitro also manifested a change from pro-inflammatory to a regulatory profile, which was achieved with a reduction of Th1 and Th17 cells, along with an increase in IL-10 and protective Treg cells [8].

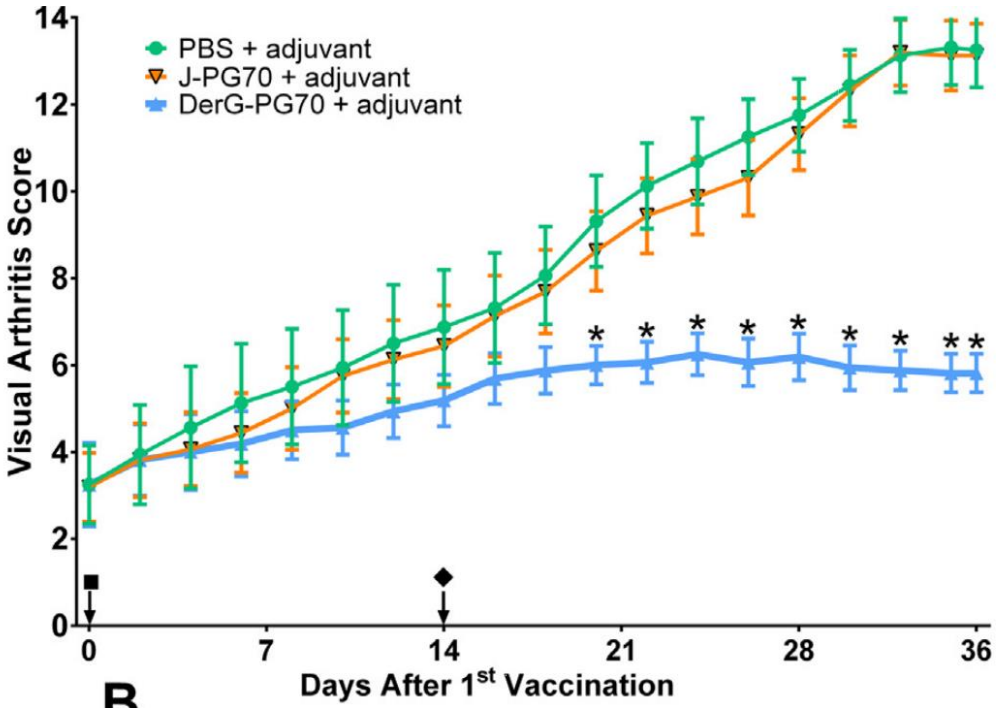

Fig 4. VA score after vaccination [8]

With the results listed above, the investigators concluded that treatment with CEL-4000 proved to generate an antigen-specific immune response in mouse models, ameliorate the RA symptoms [8]. They therefore suggest, focusing CEL-4000 therapy on the inflammatory antigen-specific immune responses, seems to manage a balance where RA symptoms are under control without losing immune protections against foreign antigens [8].

All those conclusions, however, are reached only in mouse models. As such, more future probes into therapy on human are expected in order to translate the vaccine to clinic. Moreover, failure of individual patient to respond to certain therapies is rather common in RA as the disease initiators differ from patient to patient [8]. Therefore, studies of signature T-cell cytokine phenotypes are needed to suit patients with proper individualized LEAPS vaccine therapy.

3. Conclusion

As aforementioned, therapeutic vaccines for RA, such as Rheumavax, DEN-181, and CEL-4000, prove to be a new promising therapy for the disease. Unlike traditional current mainstream treatments, they are designed according to inflammatory cytokines or autoimmune response pathways that lead to antigen-specific regulation of RA, without hurting important immune defense against foreign antigens. Vaccines like the three mentioned in this article are under development and trials at present,

with their efficacy tested from a wide range of aspects including different types of T cell number, clinical visual arthritis scores, cytokine secretion, and adverse event rates, etc., and this field is popping with new findings rapidly.

This article introduces the basic principle, experiment design, results and limitations of RA therapeutic vaccines currently under development, providing a quick overview of RA therapeutic vaccines on trials. In general, trials on the therapeutic vaccines received hopeful results, with the vaccine-treated groups lower than the control or placebo group in clinical symptom scores, autoimmune responses and so on. The reduction of inflammation and pathology of RA by therapeutic vaccines are also observed to have much higher antigen specificity than that of other drugs, which makes therapeutic vaccines the safer choice for a huge crowd of patients to whom other treatments like DMARDs do not work.

There are still, however, a range of factors holding the development of therapeutic vaccines back for the moment. For instance, problems such as the potency decline with individualization of RA, and the best way to modify antigens in the vaccines remain unanswered with current studies. More future studies on the molecular mechanism of the disease, as well as more accurate, sensible, multi-dosing assays and trials on the vaccines, are needed, in order for RA therapeutic vaccines to eventually reach the patients.

Development of RA therapeutic vaccines is of undeniable meaning, not just for RA patients who are in desperate need of a treatment with high specificity, but also for the overall understanding of vaccine design and autoimmune diseases like RA, which can be of life-changing influence for thousands of people on this planet.

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