Sole Anti-inflammatory Immunomodulators: Innovative Drugs to Prevent and Treat Autoimmune Diseases and Proteopathies

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Abstract: Objective: To review the available sole anti-inflammatory immunomodulators or adjuvants, different from pro-inflammatory ones, which elicit a Th2 immunity while inhibiting but without abrogating Th1/Th17 immunities. Adjuvants that are useful to develop vaccines for T-cell mediated autoimmune conditions.

Methods: A literature search using PubMed and Google Scholar databases was made to identify reports regarding adjuvants, mechanisms of action, pro-inflammatory autoimmunity and vaccines to treat it, immunosuppressive agents, dendritic cells, helminths, immunotolerance, and infectious diseases causing autoimmunity.

Results: Some anti-inflammatory drugs to treat autoimmune diseases inhibit DNA or protein synthesis causing global immunosuppression, which is reduced by using biologics to block key steps in the inflammatory cascade. Fucosylated glycans from helminths, which are anti-inflammatory but not immune-suppressive, offer an avenue to develop better drugs. Fucosylated glycans bind to DC-SIGN, a receptor on dendritic cells, entering the cells via receptor-mediated endocytosis, biasing their immunoresponse to a sole Th2 anti-inflammatory immunity, while inhibiting the pro-inflammatory Th1/Th17 immunities. New anti-inflammatory drugs are particular plant-derived fucosylated glycosides with immunological properties like those of helminth-derived glycans. Another class of anti-inflammatory immunomodulators is ligands of the aromatic-hydrocarbon receptor, which by activating this intracellular receptor, boosts the differentiation of regulatory T-cells, inducing an anti-inflammatory immunity. However, aromatic ligands can also stimulate a pro-inflammatory response. Exogenous aromatic ligands are usually delivered intracellularly using carriers like nanoparticles, which upon translocation to the nucleus, activate this receptor.

Conclusion: Autoimmune conditions and some infectious diseases, characterized by organ damage due to pro-inflammatory autoimmune immunoresponses, could benefit from non-immunosuppressive agents to modulate immunity; this way, averting a damaging inflammation.

Keywords: Anti-inflammatories, immunotolerance, immunosuppression, vaccines, proteopathies, autoimmunity, immunomodulation.

1. INTRODUCTION

A consequence of the changes in certain environmental factors has been the sudden increase over the last 30 years in the incidence of autoimmune diseases (ADs) in the developed countries [1]; indeed, a conservative estimate of their US prevalence places that number at 24 million cases [2]. These chronic disorders that affect predominantly women are triggered by T-cell mediated pro-inflammatory immunities, Th1 and/or Th17, against self-antigens, e.g., Rheumatoid Arthritis (RA), Multiple Sclerosis (MS), and type 1 diabetes (T1D), causing organ damage, lower quality of life and shorter lifespans [1, 2]. But ADs also carry significant financial burdens, i.e., in the US, the cost for their treatment is more than $100 billion annually, a figure that does not account for lost revenue and other indirect costs [2]. Hence, as the surge in ADs is largely occurring in the industrialized countries, these diseases can be considered a new epidemic that threatens the welfare of the developed world; a situation that calls for strategies that are cost-effective and have minimal side-effects to prevent and/or treat these conditions. Of relevance is that several studies have shown a significant coexistence of ADs and some mental illnesses, like depression [3] and post-traumatic shock syndrome [4], which is higher in women; conditions that apparently are also more prevalent in the industrialized nations [5]. Thus, currently...
available epidemiological and clinical data seem to indicate that a pro-inflammatory immune response is an underlying factor linking all of these disorders, an immunity type that apparently also plays a role in aggravating certain proteopathies like Alzheimer’s disease [6]. Proteopathies that are characterized by the abnormal refolding of certain self-antigen proteins, which leads to the formation of aberrant conformations that interfere with the protein’s normal functions, causing organ damage and detrimental pro-inflammatory immune responses. The sudden large increase of ADs and other inflammation-related diseases in the developed nations points to environmental rather than genetic factors as being responsible for their presence. Therefore, significant efforts have been dedicated to developing treatments to ameliorate these conditions, as well as strategies to prevent their onset. Efforts that have been rather successful in the treatment of some ADs, but that so far have not delivered an effective approach to prevent these conditions. The purpose of adopting these new methods is to modulate immunity in order to prevent or ameliorate ADs.

However, these immunomodulators may also have applications in the prevention and/or treatment of some infectious diseases associated with a damaging inflammatory response, which may lead to organ damage, such as a respiratory syncytial virus (RSV) [7] and the new SARS-CoV-2 or COVID-19 virus [8]; diseases that share several characteristics with T-cell mediated autoimmune conditions. For instance, both diseases are characterized by the activation of the pro-inflammatory immune Th1 and which cause organ damage, e.g., the lungs. However, in infections like RSV, the condition is aggravated by a pre-existent inflammatory anti-viral immunity elicited by a vaccine, which exacerbates the host’s inflammatory immunoresponse mounted against the virus upon infection. A situation that leads to cytokine storms and aggravation of the disease; a condition very similar to that observed with COVID-19, where the damage is caused by the inflammatory reaction against the virus [9]. A rather similar situation seems to occur with the malaria vaccine, which contains QS-21, an adjuvant that elicits both Th1 and Th17 immunities [10]. Indeed, the incidence of meningitis among vaccinated children is 10-times higher than in non-vaccinated, a situation similar to that observed with cerebral malaria [11]. Hence, it is likely that vaccines against RSV, malaria, and likely COVID-19 in the aging population, could benefit from adjuvants that induce a sole Th2 immunity but without abrogating T-cell immunity. A situation that would elicit the production of neutralizing antibodies but without stimulation of the Th1 and Th17 immunities.

1.1. The Adjuvant Notion

Traditionally, the term adjuvant has a broad connotation that includes any compound or groups of compounds that induce or enhance an immune response. This explanation needs to be updated in view of the progress during the last 30 years in innate immunity, with the discovery of the toll-like receptors (TLRs) and their ligands [12]. The identification of the different ligands has led to an understanding of how adjuvants work, like how alum, by triggering the release of uric acid, a danger signal, stimulates immunity [13]. Yet, different from TLR ligands, the immune stimulation by alum is a byproduct of the physical damage caused to the cell by its crystalline structure. Which would explain why different preparations of alum have different adjuvant activities [14]. In fact, some forms of silica have shown adjuvant activity, evidently by the same mechanism of danger signals. The same comments apply to carriers and delivery systems, e.g., liposomes and nanoparticles, structures designed to increase the targeting and delivery of antigen/adjuvant to immune cells, this way improving the immune response [15]. Frequently, these structures have ligands on their surfaces, like monoclonal antibodies (mAbs), that recognize specific receptors on some immune cells that may increase cellular uptake by endocytosis. That structures like liposomes are formed by lipid bilayers, would allow them to fuse with the cell membrane and deliver their content intracellularly for further processing [16]. Thus, while these structures have some advantages in vaccine delivery, for practical purposes, their effects as physical and without the involvement of ligand-receptor interactions with define and specific pharmacological effects. Thus, for the purpose of this review, here adjuvants will be defined as well-characterized chemical structures that upon binding to a specific cellular receptor, trigger biochemical changes that modify the immune response.

2. THERAPEUTIC IMMUNOSUPPRESSIVE DRUGS AND BIOLOGICS

A relatively successful approach to ameliorate ADs has been to prevent the pro-inflammatory immunity using immunosuppressive drugs, usually small molecules like methotrexate, cyclosporin, and prednisone, which block the synthesis of DNA and proteins needed by the immune cells to function, causing general immunosuppression [17]. While inexpensive and easy to administer, their side-effects and induced immunodeficiency increase the susceptibility to infections and cancer, thus, limiting their long-term use. Hence, new ADs’ treatments using immunomodulators known as biologics, which are essential proteins like cytokines, soluble receptors, and mAbs, that target specific steps of the immune process and have less side-effects, have been developed [17, 19].

2.1. Small Molecule Immunosuppressive Anti-inflammatory Agents

Several small-molecule compounds, like non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, cyclosporin, azathioprine, and others [17, 19], are being used to ameliorate ADs. The NSAIDs work by inhibiting the activity of the cyclooxygenase (COX), enzymes responsible for the formation of prostaglandins and thromboxane, mediators associated with inflammation [17]. Inhibition that depends on the NSAID will show some selectivity between the COX1 and COX2 enzymes. Yet, NSAIDs can also inhibit the secretion of pro-inflammatory cytokines, TNF-α, and IFN-γ by NK cells and gamma-delta T-cells, inhibitions that are independent of the COX enzymes’ inhibition [20]. Indeed, this pattern of inhibitions may explain the apparent changes in immunity towards a Th2 type, which may aggravate infectious diseases [20], but can be beneficial in autoimmune conditions and wound healing [21]. However, it is doubtful that inflammation and its sequelae of conditions, may be
that epidemiologically, the incidence of Alzheimer’s seems to be lower in the less developed countries, which points to some environmental factors, like the high incidence of parasitic infections in those countries [31], which indicates a role for the immune tolerance induced by these parasites, similar to that observed with ADs [32]. However, that the incidence of Alzheimer’s disease is expected to drastically increase during the next 30 years in Latin America and Asia while stabilizing or decreasing in the developed countries [33], points to some other factors. Such as a significant increase in their aging population due to better public health care, which reduced the impact of infectious diseases and promoted better nutrition during the past few decades. Yet, the negative correlation between infections by parasitic helminths and the incidence of Alzheimer’s disease observed around the world strongly suggests a role of anti-inflammatory immunity in either preventing or delaying the onset of the neurodegenerative disease. A situation that would change as the parasitic infections are eradicated as a result of better public health.

2.3. Biologics as Anti-inflammatory Therapeutic Agents

Because biologics target specific ligands or receptors that are critical for the induction of inflammation and frequently are modifications of products naturally found in the body, they can have a more limited inhibitory action, which decreases the side effects observed with the small molecule immunosuppressive drugs. Hence several biologics have been approved for the treatment of ADs, like those against the pleiotropic cytokine tumor necrosis factor (TNF), which plays a critical role in the inflammatory response. Biologics that include soluble TNF-receptor fusion proteins and anti-TNF mAbs [17, 18], proteins that bind with high affinity to TNF, and in this way blocking its access to the natural cellular receptor. Other biologics avert inflammation by blocking the co-stimulatory signals required for T-cell activation and rendering it anergic, like a chimeric molecule having the CTLA-4 receptor. This chimeric receptor binds with high affinity to the CD80/86 ligands on dendritic cells (DCs), blocking their interactions with the CD28 receptor on T-cells [19], which leads to T-cell anergy and attenuation of the overall immune response [20]. In fact, these biologics act as immune checkpoint inhibitor drugs. Other successful biologics to treat ADs are mAbs that block specific cytokines or their receptors involved in the autoimmune response, like those against TNF and IL-23, which block the cytokine cascade that leads to a damaging pro-inflammatory immunity and organ damage [17].

3. ACTIVE IMMUNOTHERAPY TO PREVENT INFLAMMATION

While biologics have advantages over immunosuppressive drugs, they are significantly more expensive, require constant treatments, and their administration frequently entails special personnel and facilities. Besides, due to their MOA, the induced immunosuppression can sometimes be rather “global,” as they block key steps in the inflammatory process [17]. Therefore, safer methods to induce immunotolerance while limiting immunosuppression are being pursued. Methods that target the self-antigens are responsible for autoimmunity and involve strategies like co-administration of antigens and immune suppressive drugs [34, reviewed in 35]
and tolerogenic vaccination or active immunization. Indeed, several proteins that are related to self-antigens and induce T-cell mediated autoimmune responses have been identified. Proteins that may share common structures with certain pathogens, which upon infection may induce an immunological cross-reactivity that triggers autoimmunity by a mechanism called molecular mimicry [36,37]. Otherwise, self-antigens may undergo post-translational modifications, like methylation and citrullination, creating neoantigens that elicit a cross-reactive immune response with normal self-antigens, generating autoimmune conditions like RA [19,38]. Alternatively, a self-antigen may be altered via mutations to yield neoantigens that cross-react with the normal self-antigen. But, all of these conditions may be worsened by the process called epitope-spreading, which allows the development of damaging immune responses against other epitopes that are different from the initially targeted ones, a frequent occurrence in chronic ADs [36-38].

3.1. Antigen-specific Immunotherapy – Co-delivery

An effective strategy in allergy therapy has been to induce an antigen-specific immune response, i.e., antigen-specific immunotherapy (ASIT), which elicits immune tolerance for specific allergens [35]. An approach that is now being applied preclinically to ADs by administering antigens along with immunomodulatory agents, i.e., co-administering or co-delivering self-antigens with agents, like the small immune-suppressive compounds 2-(1H-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester, dexamethasone, and others [34,35], to immune cells. An alternative approach is to deliver a self-antigen with an immunomodulatory protein, like IL-4, usually as a co-encoded plasmid DNA vaccine [35]. A comparison of the co-administration of an antigen with an immunomodulator as a mixture, with their co-delivery using carriers as nanoparticles and liposomes having both components, shows that the co-delivery approach is more effective [35,39], as a result of both components being delivered to the same Antigen-Presenting-Cell (APC), i.e., DCs and macrophages; a delivery that may be increased by using ligands on the carrier that target APC’s surface receptors. Actually, co-delivery does not always require a carrier like nanoparticles since similar effects may be achieved if the antigen and immune modulator can form complexes that are held by either covalent bonds or hydrophobic-ionic interactions [35], which can be delivered to the same APC. A situation that is evident with Quillaja saponin-derived adjuvants, like QS-21, where complexes formed by strong hydrophobic interactions between the saponin and proteins, like ovalbumin or bovine serum albumin, induce a strong pro-inflammatory immunity with the formation of cytotoxic T lymphocytes (CTL) [40]. Also, if the binding by hydrophobic-ionic interactions between a saponin and a protein, like QS-21 and lysozyme, is not strong enough to allow the formation of an immune stimulatory complex, it is possible to covalently link the protein to the saponin to yield a stable construct, which is capable of entering and being processed by the APCs to deliver a pro-inflammatory immune response [40]. Since the adjuvant decides the type of immunity, a Th2 immune response would be obtained by using an anti-inflammatory saponin rather than a pro-inflammatory one like QS-21; a situation that provides the basis to develop vaccines to prevent and/or treat ADs.

A consequence of the co-delivery of a self-antigen with an anti-inflammatory immune modulator is the stimulation of secretion of Th2 cytokines like IL-10, and in some cases, the restoration of the balance between the antigen-specific regulatory T-cell (Treg) and autoreactive effector T-cell (Teff) populations. Indeed, Treg cells, by suppressing Teff cells, reestablish immunotolerance while preventing damage to T-cell mediated autoimmune responses [41]. Therefore, immunotolerance is the result of actions, independently or coordinated, of regulatory DCs (DCreg), which respond to a variety of exogenous and endogenous stimuli, and Treg cells [reviewed in 42]. Thus, the available information shows that DCs serve as a critical switch between immune activation and immune tolerance, i.e., they have a bidirectional role in either provoking or preventing ADs [42]. Moreover, DCs have a critical role in self-antigen uptake, processing, and presentation, which is important to understand in order to gather how immune tolerance is broken. Antigen uptake may occur by phagocytosis, receptor-mediated endocytosis, and micropinocytosis, which leads to its processing in the DCs for presentation to T cells. In effect, C-type lectin receptors (CLRIs) on DCs play a critical role in the uptake of glycosylated antigens, with the glycan’s composition biasing their processing toward the MHC-I or MHC-II processing pathways to yield a CD8+ or CD4+ T-cell immune response, respectively [43]. Hence, DCs are unique targets for preventive and/or therapeutic intervention in ADs.

4. ANTI-INFLAMMATORY AGENTS FOR THE PREVENTION/TREATMENT OF ADs

Considering the vaccines’ success in preventing infectious diseases, the use of self-antigen-targeted vaccines to avert and/or ameliorate autoimmune diseases seems a viable option. Different from the pro-inflammatory Th1/Th17 immune responses induced by infectious disease and cancer vaccines, those used to prevent and/or treat autoimmunity must induce a sole anti-inflammatory Th2 immunity and restore tolerance; an endeavor difficult to achieve because of the scarcity of anti-inflammatory immunomodulators; apparently, a result of Th2 immunity being an immunological newcomer as compared to the Th1/Th17 pro-inflammatory immunities [44]. Hence, in contrast to a large number of pro-inflammatory immunomodulators and cell receptors, e.g., toll-like receptors (TLRs) and their ligands, those related to Th2 immunity are limited. Alum and oil/water emulsions, the traditional Th2 vaccine adjuvants, have several properties characteristic of pro-inflammatory modulators, as discussed later. Two receptors associated with Th2 immunity are the aryl hydrocarbon receptor (AhR) and the lectin receptor DC-SIGN. Both receptors, depending on the nature of their ligands, may induce either a Th1/Th17 or a sole Th2 immunity. Of importance is that while the Th1/Th17 pro-inflammatory immunities are always followed by the Th2 anti-inflammatory response, the latter can exist by itself; the reason being that Th2 immunity is a rapid repair mechanism to heal tissue damage caused by pro-inflammatory immunities.

An important vaccine development area maybe that concerning pathogens that can trigger severe pro-inflammatory
responses, causing organ damage. Indeed, their induced immune responses share certain traits with T-cell mediated autoimmune conditions, like inducing Th17 immunity, that lead to organ damage [7, 8]. This is relevant for vaccine development since a post-vaccination exposure to the pathogen may induce an uncontrolled damaging pro-inflammatory response, like in the cases of RSV, malaria, and likely COVID-19 [7, 8, 11]. The RSV case is a well-known case that has been thoroughly reviewed [45]. A conclusion of that study is that the neutralizing antibodies against RSV alone cannot explain the antiviral protection. Indeed, the authors stress the role of the glycosylation of the Fc-region in the antibodies Fc-mediated effector functions [45]; functions that apparently play a crucial role in both protection and aggravation of the disease, a role that is being ignored. While it has been proposed that adjuvants may improve the antiviral protection [46], their role in the immune response, including the Fc-glycosylation, has not been addressed. Importantly, since an increasing body of evidence shows that the initial immune response affects the glycosylation process, the immune response induced by DCs, shapes the Fc-glycosylation patterns toward a pro or anti-inflammatory effector [47]. Therefore, anti-inflammatory adjuvants may have a crucial role in some infectious disease vaccines, including a COVID-19 vaccine for the elderly. An anti-inflammatory immunity by preventing delivery of inflammatory stimuli to B-cells would prevent the formation of glycan patterns that favor the antibodies’ inflammatory effector functions [47]; in this way, impeding the induction of secondary inflammatory processes mediated by the interactions of antibodies’ Fc-regions and their corresponding Fc-receptors (FcRs) on the different immune cells.

5. AHR LIGANDS AS ANTI-INFLAMMATORY MODULATORS

The AhR is a ligand-activated transcription factor located in the cytoplasm that is found in various immune cells, which upon activation by a ligand, translocates into the nucleus to control the transcription of several target genes [48]. The roles of AhR are diverse, and its activation by some ligands, which are characterized by the presence of aromatic rings, may lead to immune suppression. This situation may be explained by a boosted Treg cell differentiation, yet, AhR activation can also induce Th17 polarization, which aggravates ADs [49]. Because AhR is a cytoplasmatic receptor, its activation will require intracellular delivery of the ligands either alone or co-delivered with a self-antigen, an objective that may be achieved by using liposomes, nanoparticles, and other delivery systems [34, 35, 39]. An improvement of this strategy, i.e., covering the carrier with agents like antibodies, to target specific groups on the APCs, results in enhancements of selectivity and carrier uptake by these cells. Based on the immune suppression induced by some AhR’s ligands, their development as anti-inflammatory agents and/or adjuvants in vaccines to ameliorate ADs are being evaluated.

Therefore, a new family of immune-suppressive drugs is comprised of certain ligands of AhR, a receptor characterized by its ligand promiscuity that can bind a diversity of compounds carrying cyclic aromatic structures [48-50]. Indeed, many of the known anti-inflammatory AhR ligands are phytochemicals like flavonoids, resveratrol, and curcumin [50]. The potential problem is that the structural characteristics of these ligands, which would determine their agonistic or antagonistic role, are still being elucidated. Indeed, it is apparent that dissimilar AhR ligands may bind at different sites within the ligand-binding domain [51]; a situation that complicates the design of new agonists or antagonists. Indeed, recently the application of high throughput screening methods has allowed to identify the already approved anti-inflammatory drug ifelunomide as a human AhR agonist [52]. Thus, while promising, the broad use of AhR ligands in products like vaccines for ADs may need additional work to establish their structure-activity relationships (SARs) and MOAs, as well as their safety profile, all required in the development of new preventive and/or therapeutic products.

6. DC-SIGN LIGANDS – IMMUNOMODULATORS

There is significant evidence that induction of a strong Th2 immunity improves ADs, i.e., during pregnancy, conditions like MS and RA go into remission, only to relapse post-partum [53]. Indeed, it has been proposed that during pregnancy, there is a shift from Th1 to Th2 immunity, which results in stronger humoral immunity and a decreased T-cell mediated immunity, but without immunosuppression [54]. Another well-known condition associated with pregnancy is post-partum depression, which may occur at any time after childbirth [55], a complex condition that has been assumed to be a result of hormonal changes and perhaps an inflammatory response. A characteristic of pregnancy is the increased fucosylation of different glycoconjugates, which after childbirth returns to their normal levels; glycosylation changes correlate well with those observed for ADs [56]. However, the relations between fucosylation and anti-inflammatory activity are intricate. For instance, it has been reported that in a mouse model, inhibition of terminal fucosylation results in an anti-inflammatory M2 macrophage phenotype and suppression of type II collagen-induced arthritis [57]. Nonetheless, support for the role of fucosylation in biasing the immune response toward Th2 immunity is given by the fact that similar immunological changes affecting the course of ADs occur during infection by parasitic helminths, where many T-cell mediated ADs improve. Apparently, a result of the fucosylated glycans mimicking LewisX antigen, like lacto-N-fucopentaose III (LNFPIII) (Fig. 1), that the parasites produce to prevent damaging pro-inflammatory responses by the host [58]. A strategy also used by some tumor cells, which produce fucosylated glycans to inhibit the pro-inflammatory immunity that would destroy them while promoting a harmless anti-inflammatory immunity [59]. The capability of the fucosyl residue to induce an anti-inflammatory immunity is explained by its interactions with the DC-SIGN on DCs; a CLR that can bind either mannose or fucose, biasing DCs to a Th1 or Th2 immunity, respectively [60]. Thus, glycans carrying fucosyl residues with free hydroxyl groups at positions 3 and 4, which would allow interactions of this sugar with certain amino acid residues at the DC-SIGN’s binding site, can be effective immunomodulators capable of biasing DCs to induce a systemic anti-inflammatory immunity as well as adjuvants of new anti-inflammatory vaccines [60-63].
anti-inflammatory drugs, but without being immunosuppressive. Compounds that interact with DC-SIGN may also be used as their induced systemic Th2 immunity, these fucosylated glycans up-regulate the production of IL-10 and Treg cells [64], while others mention that it does not change the frequency of these regulatory T cells significantly [65].

6.1. Fucosylated Glycans - Anti-inflammatory Modulators

Because of the apparently “beneficial” effects of helminth infections on ADs, as shown by the low incidence of these diseases in areas where helminthic parasites are common, their use has been proposed to treat these conditions; an unlikely practice due to public health hazards. Besides, the immunomodulatory compounds produced by these parasites that are responsible for biasing immunity and inducing immunotolerance have been identified and their MOA has also been elucidated, eliminating the need for “helminth therapy.” In fact, those compounds provide the basis to identify and design new anti-inflammatory agents, like LNFPIII [58, 63] and QT-0101 [61, 62] (Fig. 1). Hence, the application of medicinal and natural products chemistries is allowing the development of safe, cost-effective, and relatively easy to produce anti-inflammatory modulators for the prevention and treatment of ADs, as compared to biologics. Because of their induced systemic Th2 immunity, these fucosylated compounds that interact with DC-SIGN may also be used as anti-inflammatory drugs, but without being immunosuppressive, i.e., they will inhibit, but without abrogating Th1 immunity, which is critical to prevent infections and cancer. Indeed, these compounds will modulate the immune system to produce a Th2 immune response similar to the one observed during pregnancy or helminthic infections, inducing immunotolerance but without causing immunosuppression. However, the effects of FLNPIII on the Treg cell population are still unclear, with some reports indicating that this fucosylated glycan up-regulates the production of IL-10 and Treg cells [64], while others mention that it does not change the frequency of these regulatory T cells significantly [65].

6.2. Fucosylated Ligands as Vaccine Adjuvants

A major obstacle to the development of anti-inflammatory vaccines is the number of sole anti-inflammatory Th2 adjuvants, which is minimal compared to those of pro-inflammatory Th1 adjuvants; apparently, a result of the Th2 immunity’s late appearance during evolution [44]. Although alum has been traditionally assumed to be a sole Th2 adjuvant, a conclusion derived mainly from infectious disease vaccines where inactivation of pathogens may occur by neutralizing antibodies and/or cell-mediated immune responses, and recent studies have also questioned this assumption. Indeed, alum’s complex MOA involves induction of Th1 immunity, as shown by the production of pro-inflammatory cytokines, complement activation, and activation of monocytes [66, 67]; all inflammatory processes that would improve the efficacy of infectious disease vaccines but would be damaging for autoimmune vaccines, as it seems to have been the case [68, 69]. Indeed, alum, besides performing poorly in the elderly, the population most affected by ADs, in humans, induces a Th1/Th2 immunity [70]. Therefore, the adjuvants capable of inducing a sole systemic Th2 immunity appears to be limited at present to three groups. The first group is composed of oligosaccharides carrying terminal fucosyl residues, e.g., LewisX trisaccharide and LNFPIII, found in erythrocytes and helminths, respectively [58]; while the second group includes triterpene saponins, having fucosyl residues, e.g., QT-0101 [61]. The third group is composed of certain AhR ligands, as previously discussed [49] (section 5). While the first two groups, due to their fucosyl residues, bias DCs to an anti-inflammatory immunity, the saponins’ MOA is more complex [71]. Saponins, due to their amphipathicity, can form complexes with protein antigens that are co-delivered, entering DCs by endocytosis and being processed into peptides at the endosome-lysosome compartment. Subsequently, the saponins intercalate into the cholesterol-rich endosomal membranes, disrupting them and allowing the peptides to escape into the DCs’ cytosol for processing by the exogenous pathway and eventual production by B-cells of antibodies against those antigens [71]. Hence, immunogenic vaccines having self-antigens and fucosylated ligands as adjuvants would induce switching from the damaging pro-inflammatory immunity characteristic of ADs to a safe anti-inflammatory, humoral immune response [72]. Because several self-antigens and the genetic risk factors associated with some ADs are known, it is possible to foresee situations where immunogenic vaccines would be

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**Fig. (1).** Structures of fucosylated immunomodulators. LNFPIII (a) and QT-0101 (b). In QT-0101, the fucosyl residue is linked to a triterpenoid nucleus and an oligosaccharide chain at positions 1 and 2, respectively, but leaving free the hydroxyls at positions 3 and 4, critical for binding to the DC-SIGN receptor. Chemical structures were drawn using software from ChemDoodle, version 8.1.0, iChemLabs.
used in a preventive rather than therapeutic mode, minimizing the risk of organ damage.

A commonly ignored fact in vaccine development is that the adjuvant decides the type of immune response, i.e., Th1/Th17 or Th2, as well as its magnitude and immunological memory, regardless of the protein antigen. Indeed, protein antigens lacking T-cell epitopes, but formulated with Th1 adjuvants, will still elicit a systemic pro-inflammatory immunity mediated by Th1 cytokines; an undesirable situation in ADs [72]. Moreover, a systemic Th1 immunity would favor the production of antibody or immunoglobulin (IgG) glycoforms, which upon binding to the Fc receptors (FcγR) on different immune cells, e.g., DCs and macrophages, would act as effectors of inflammation, inducing their activation and subsequent inflammatory process [73]. However, under anti-inflammatory conditions, the Fc region’s glycans would show the presence of terminal sialic acid residues with an α2,6 linkage, which hinders the IgGs binding to FcγRs, blocking their capacity to induce inflammation [56, 74]. That under non-inflammatory conditions, the Fc sialylation process can take place either at the B-cell or after IgG secretion [75], stresses the importance of a sole systemic anti-inflammatory immunity. A requirement that has not been fulfilled by any immunogenic vaccine for ADs or protozoan infections; however, it is apparent that such a condition may extend to some antiviral vaccines, like RSV and likely COVID-19.

6.3. Fucosylated Ligands as Anti-inflammatory Drugs

It is evident from the systemic Th2 immunity, occurring either during pregnancy or after infection by helminths where free unconjugated fucosylated compounds that can bind DC-SIGN are capable of biasing DCs toward a non-specific systemic anti-inflammatory Th2 immunity, inducing tolerance [63, 76]. Actually, this generic immunity would explain why the beneficial effects observed in the two populations mentioned above extend to various ADs rather than some selected ones. This situation strongly indicates that fucosylated ligands like LewisX antigen, LNFPIII, and QT-0101 (Fig. 1) can be used as potential anti-inflammatory drugs. Although significant efforts have been dedicated to developing non-carbohydrate glycomimetic DC-SIGN antagonists as anti-infectious agents [77, 78], considerably less efforts have been dedicated to developing fucose-based anti-inflammatory agents. Indeed, most anti-viral agents are based on sugar mannosse, which is more common among pathogens than fucose. While there is some information about these compounds’ antagonistic effects on the infection process, there is almost none about their modulatory effects on the induced immune response. Yet, since fucose-based glycomimetics are more stable than the natural ligands and bind in a similar manner, it is expected that they would bias DCs to Th2 immunity or tolerance. Some methods have been developed to prepare synthetic fucosyl analogs, i.e., one uses a shikimic acid-derived scaffold with the necessary arrangement of hydroxyl groups [79] (Fig. 2A), that can act as DC-SIGN agonists or antagonists of viral infection and interact with the same side-chain residues in the binding site as fucose does [80]. From known X-ray structures of DC-SIGN-LewisX complex, several ligands that use an α-fucosylamide anchor have been designed [81] (Fig. 2B), some carrying a cyclic cis-β-amino acid linker, which shows a higher affinity than the native ligand LewisX [82]. That DC-SIGN binds different ligands, indicates the plasticity of its binding site, which would explain the capacity of compounds like QT-0101, a triterpene glycoside, to interact with this lectin receptor and induce a Th2 immunity [62]. These reports indicate the potential therapeutic applications of fucose and fucose-related derivatives as anti-inflammatory drugs, which would act at the DC level rather than at some later step in the Th1 cytokine inflammatory cascade, preventing immune suppression.

Fig. (2). Structures of fucose glycomimetic ligands. The moieties equivalent to the fucosyl residues in the non-carbohydrate analogs from a shikimic acid-derived frame (A) and an α-fucosylamide (B) are shown here. For comparison, the positions of the relevant hydroxyl groups in the shikimic acid derivative (A) are shown in relation to those from fucose (C). Adapted from the previous studies ([79] and [81]), chemical structures were drawn using software from ChemDoodle, version 8.1.0, iChemLabs.

7. IMMUNOGENIC VACCINES IN AUTOIMMUNE DISEASES

Those ADs that may be prevented/treated by immunogenic vaccines, e.g., RA, MS, and T1D, are characterized by a T-cell pro-inflammatory response against self-antigens. While AD tolerogenic vaccines by eliciting an immune response that deletes the T-cells causing the autoimmune reaction against a self-antigen have delivered promising preclinical results [72], little success has been achieved with immunogenic vaccines [69]. However, since these immunogenic vaccines contained adjuvants, like alum or oil/water emulsions, derived from vaccines against pathogens where the optimal immunity would be pro-inflammatory, their poor performance may be significantly improved by the use of sole Th2 adjuvants. Nonetheless, while tolerogenic vaccines could be effective as therapeutics, due to their MOA, they may not be as effective prophylactically since deletion of the
lysosomal compartment to induce an antigen-specific anti-inflammatory immune response [58, 84, 85].

T-cells liable for the immune response against a self-antigen would occur only after autoimmunity has been established. In contrast, immunogenic vaccines may induce a primary or Th2 immunity against a self-antigen, this way preventing a damaging Th1 inflammatory response. Indeed, co-delivery of DCs of the antigen and fucosylated ligand, in the presence of lipid A, leads to an antigen-specific Th2 anti-inflammatory response, with inhibition of the pro-inflammatory Th1/Th17 immunoresponses (Fig. 3). A potential treatment that may require regular immunizations to maintain the anti-inflammatory immune response against the specific antigen, an acceptable practice considering that these vaccines would not be immunosuppressive. Indeed, it has been shown that DCs activated by the fucosylated ligand LNFPIII induced CD4+ Th2 immune responses, but without impairing CD8+ memory and effector cytotoxic T-lymphocytes (CTL) responses, i.e., they did not show immune suppression [83]. The primary process that takes place at the DC level is shown by the fact that covalent linkage of a protein antigen to LNFPIII forming a neoglycoconjugate is essential to show adjuvant activity [58, 72]. Thus, it is evident that the initial binding of the fucosyl residue to DC-SIGN mediates the receptor-mediated endocytosis of the linked protein antigen and its processing at the DC’s endolysosomal compartment to induce an antigen-specific anti-inflammatory immune response [58, 84, 85].

An AD preventable and treatable with immunogenic vaccines would be T1D, which starts during childhood or early adulthood and is characterized by the destruction of pancreatic β-cells that produce insulin [69]. While its cause is unknown, there is evidence that genetic risk factors combined with environmental ones trigger this disease. In the US, T1D affects 1.25 million people, but its rapid increase points to environmental factors as being the main reason. While currently there are no ways to prevent T1D, some vaccines containing self-antigens, like pro-insulin, glutamic acid decarboxylase 65 (GAD65), and a peptide derived from Heath Shock Protein 60, have been tested clinically. Yet, despite promising preclinical results, clinical studies have been disappointing and uncertain [69]. While the failure of translating promising results from animal models to humans may raise questions about the models’ validity, there are also issues concerning these vaccines, like their adjuvants coming from infectious disease vaccines, which require a pro-inflammatory immunity. As previously indicated, although alum has been assumed to induce only Th2 immunity, there is evidence that it also induces activities related to Th1 immunity [66, 67], i.e., in humans, alum is considered to be a Th1/Th2 adjuvant [70], a situation that while advantageous in infectious disease vaccines, would be aggravating in ADs immunogenic vaccines. Also, the alum’s effects on the Treg cell population are unclear and apparently affected by the delivery route, dose, and other factors such as genetic ones, as shown by the induction by alum-containing vaccines of allergies in certain populations [86, 87]. Hence, it would be valuable to test those self-antigens with fucosylated ligands as adjuvants that, by acting at the DC level, would bias the immune system to a sole Th2 immunity while inhibiting, but without abrogating Th1 immunity [61, 72, 83].

It is likely that similar problems to that of the GAD65 vaccine occurred with other vaccines, like those against RA and MS, diseases that during pregnancy go into remission.

Fig. (3). Effects of fucosyl (Fuc) ligands on the immunity. Fuc. ligands like LNFPIII or QT-0101, depending on their association with an antigen (Ag.*), may induce an antigen-specific immune response. Fuc residues upon binding to the lectin receptor DC-SIGN, located on the dendritic cell (DC) surface, acting in conjunction with TLR4, activates kinase MK2, leading to phosphorylation (P) of the DC-SIGN associated LSP1. Inactivation of CYLD results in ubiquitination of Bcl3 (Ub-Bcl3), which translocates to the nucleus increasing expression of the anti-inflammatory cytokine IL-10 and Th2 chemokines CCL17 and CCL22, while suppressing pro-inflammatory cytokines IL6, IL12, IL23 [60, 63]. Cytokine changes in the presence of IL4 [60, 63], activates and biases the DCs toward an anti-inflammatory phenotype. Indeed, it induces the production of Tregs as well as immune tolerance, which is therapeutically beneficial in autoimmune diseases (AD) and some mental illnesses. The administration of the Fuc ligand linked either covalently or by non-covalent interactions to an Ag.* would allow uptake of the Ag by endocytosis mediated by DC-SIGN, its processing by the DC, and the stimulation of Th2 immunity with the production of antibodies against that Ag; an immune response that may be prophylactically and therapeutically beneficial in AD and certain proteopathies like Alzheimer’s disease.
speculate that ADs immunogenic vaccines containing self-antigens with sole Th2 fucosylated adjuvants would elicit effective anti-inflammatory, immune responses for preventive and therapeutic purposes. Considering these vaccines' MOA, it is possible to speculate that combinations of immunogenic and tolerogenic vaccines can be more effective in the long-term. While immunogenic vaccines can be used in both preventive and therapeutic modes, particularly if there are known genetic risk factors, the tolerogenic vaccines may be quite effective as therapeutics. Indeed, tolerogenic vaccines by inducing an immunity that eliminates in an antigen-dependent manner those T-cells that are responsible for the AD, may have longer-term therapeutic effects than the immunogenic ones [7]. Although vaccines can be a safe and economical option to prevent and/or treat T-cell mediated ADs, their efficacy may be somewhat diminished by the immune decline associated with aging and the concomitant increase in inflammatory immunity; a situation that could be alleviated by the use in the elderly population of biologics, whose efficacies are not altered by immunosenescence.

A special case is the Alzheimer’s disease, a proteopathy for that, at present, there are no ways to prevent or treat it. Based on the amyloid-β (Aβ), hypothesis efforts have been made to develop vaccines and mAbs, to ameliorate it. Yet, all of the vaccines have failed due to the use of the wrong antigen, i.e., normal monomeric Aβ, rather than the cytotoxic oligomeric Aβ, plus the wrong adjuvant, i.e., pro-inflammatory ones like QS-21 [88]. A situation complicated by the fact that the objective was to treat rather than prevent Alzheimer’s disease. While the search for effective mAbs has yielded mostly failures, the mAb aducanumab, which targets the cytotoxic oligomeric Aβ, can somewhat slow down the onset of disease, as reviewed in a previous study [89]. Results that do not support aducanumab use as a therapeutic agent may support its use to slow the onset of disease; an expensive proposition. The suboptimal performance of this mAb may be due to the fact that it only neutralizes some of the cytotoxic Aβ oligomers. Yet, the results indicate that a well-designed vaccine that elicits an anti-inflammatory immunity may boost the natural protective immunity against a broad population of oligomeric Aβ, slowing down and/or preventing the onset of Alzheimer’s disease [89].

8. GENETIC-THERAPEUTIC ENGINEERING IN AUTOIMMUNE DISEASES

The hope for a cure for autoimmune conditions has propelled the use of genetic editing as a viable alternative to treat these diseases [90, 91]. Yet, while the risk versus benefit of genetic editing is justified in terminal cancers and diseases caused by genetic defects, at present, it cannot be justified for autoimmune conditions [92]. Autoimmunity, the result of the immune system mounting a pro-inflammatory immunity and attacking the body’s own cells, is typically not due to genetic factors. But it is initiated by external agents, like viruses and chemicals, which cause cell damage, exposing self-antigens that normally are hidden to the immune system. Alternatively, self-antigens are chemically modified to yield neoantigens that cross-react with the self-antigen [36, 38]. Nonetheless, some genetic factors may increase the susceptibility to autoimmune conditions after external agents have revealed and exposed critical self-antigens to the immune system or have created neoantigens. However, mutations at the LRBA gene [93], which encodes for a protein critical for the processing and trafficking of the CTLA-4 receptor, an immune checkpoint, or the CTLA-4 receptor itself [94], would lead to autoimmunity. Hence, the mutated LRBA gene is a strong candidate for the use of genetic-therapy methods.

Some therapeutic genetic products under consideration are those based on CRISPR gene editing, which supposedly in a precise manner remove certain DNA sequences and replaces them with alternative ones. To add precision, the cutting enzyme Cas9 is assisted by certain RNA sequences [95]. Hence, it has been proposed that with this technique, it is possible to treat a variety of diseases, which presumes that the precision of DNA editing is exceptionally high. Studies have shown that gene editing alters the genetic makeup of an individual by causing mutations and genetic damages [96-98], which frequently cannot be detected by the customary DNA tests. Indeed, recently it has been reported that CRISPR can create unwanted duplications that cannot be detected by PCR analysis [99]. These changes would increase the risk of cancer, in addition to the production of cells with damaged genetic information, which may lead to unknown diseases that may be worse than the ones being cured. Also, because of the ex vivo processing and need for hospitalization, the cost of these treatments would be significantly higher than the current ones. Hence, genetic editing methods, while promising, are at too early development stage to be considered as alternative treatments of commonly known autoimmune conditions and proteopathies in the near future due to the associated risks.

CONCLUSION

It is evident that large-scale prevention and treatment of the rapidly increasing ADs, certain proteopathies and mental illnesses, would require the induction of Th2 biased anti-inflammatory immunity, like that occurring during pregnancy and helminths infection, rather than immunosuppression. While there are several small-molecule compounds as well as large biologics with anti-inflammatory properties, most have side effects, are immunosuppressive, difficult to administer, and some like the biologics are very expensive [100]. However, none of the currently available drugs take advantage of the fact that the fucosyl residues present in various natural ligands, upon binding to the DC-SIGN receptor, safely bias the DCs toward a sole systemic anti-inflammatory Th2 immunity without immunosuppression [83]. A process that depends on various factors may elicit either a non-specific or specific Th2 immune response, but without abrogating the Th1 or Th17 immune responses, which are critical for the defense against infectious agents and tumor cells. Thus, the available fucosylated Th2 immunomodulators, LNFPIII, and QT-0101, may be suitable candidates for use alone as anti-inflammatory drugs in conditions like depression [101] and autoimmune conditions. However, they may also be useful if formulated with specific immunogens as vaccines to prevent and/or treat autoimmune diseases and some proteopathies, like Alzheimer’s disease [72, 89].
Because fucosylated ligands are not immunosuppressive and induce CD4+ Th2 immune responses, but without impairing CD8+ memory and effector cytotoxic T-lymphocytes (CTL) responses [83]; they may be of value in certain infectious disease vaccines; an application that under certain conditions may be used in vaccines against pathogens that elicit a damaging pro-inflammatory immunity in the host, e.g., RSV and likely COVID-19; an immune response that frequently under natural conditions has the ability to damage Th17 in addition to Th1 [8,9]. Hence, it is feasible to propose that, like with T-cell mediated autoimmunity, an initial Th1 immunoresponse against an antigen may be modulated to an anti-inflammatory Th2 immunity with the production of antigen-specific antibodies [72] by subsequent immunizations with the fucosylated adjuvants LNFPIII [84] or QT-0101 [10]: this way, averting undesirable pro-inflammatory immune responses, but without immunosuppression. A strategy of interest in vaccines against diseases like COVID-19 for the elderly population, where a damaging pro-inflammatory immunity is the cause of severe disease and frequently death.

Furthermore, taking advantage of drug design strategies and medicinal chemistry methods, it would be possible to develop synthetic molecules to specifically modulate the DC’s functions, a situation that would allow the introduction of new therapies for ADs, which would also be cost-effective; a relevant issue, considering the significant increases in the health care cost, which are a result of new but expensive treatments. In fact, taking advantage of the currently available information concerning these fucosylated ligands’ SARs and MOAs, unique non-carbohydrate glycomimetic have been developed that can act as DC-SIGN agonists or antagonists [79]. These new compounds provide the basis to design new anti-inflammatory drugs with improved properties to fulfill the needs created by these rapidly emerging epidemics. A lesson derived from innate immunity is that the stimulation of different TLRs may result in agonistic or antagonistic effects [102]. Hence, it would be of interest to explore the possible existence of those effects between DC-SIGN and AhR, receptors that induce an anti-inflammatory immunity and apparently influence the production of Tregs.

CONSENT FOR PUBLICATION
Not applicable.

FUNDING
None.

CONFLICT OF INTEREST
The author is President and Chief Scientific Officer of Qantu Therapeutics, Inc. and has issued patents covering the use of de-acylated saponins as Th2 adjuvants in vaccines against proteopathies. He also has filed patent applications covering the use of de-acylated saponins in vaccines against coronaviruses.

ACKNOWLEDGEMENTS
Declared none.

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