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Addressing the key issue: Antigen-specific targeting of B cells in autoimmune diseases

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Autoimmune diseases are heterogeneous pathologies characterized by a breakdown of immunological tolerance to self, resulting in a chronic and aberrant immune response to self-antigens. The scope and extent of affected tissues can vary greatly per autoimmune disease and can involve multiple organs and tissue types. The pathogenesis of most autoimmune diseases remains unknown but it is widely accepted that a complex interplay between (autoreactive) B and T cells in the context of breached immunological tolerance drives autoimmune pathology. The importance of B cells in autoimmune disease is exemplified by the successful use of B cell targeting therapies in the clinic. For example, Rituximab, a depleting anti-CD20 antibody, has shown favorable results in reducing the signs and symptoms of multiple autoimmune diseases, including Rheumatoid Arthritis, Anti-Neutrophil Cytoplasmic Antibody associated vasculitis and Multiple Sclerosis. However, Rituximab depletes the entire B cell repertoire, leaving patients susceptible to (latent) infections. Therefore, multiple ways to target autoreactive cells in an antigen-specific manner are currently under investigation. In this review, we will lay out the current state of antigen-specific B cell inhibiting or depleting therapies in the context of autoimmune diseases.

1. B cells in autoimmune disease

The genetic pathways that diversify the antigen receptor repertoire of B and T cells are essential for a healthy and versatile immune system. To maintain immune homeostasis and avoid autoimmunity, various mechanisms of immunological tolerance eliminate, edit or neutralize cells that bind to self-antigens outside the window of proper affinity [1,2]. Autoimmune diseases (AIDs) are multifactorial diseases to which genetic predisposition (such as the Human Leucocyte Antigen (HLA)--system) and encountered environmental factors contribute significantly [3,4]. Examples include rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS) and type 1 diabetes (T1D), which can affect a diverse set of tissues such as the joints, kidneys, central nervous system or pancreas. AIDs are a major and growing cause of morbidity and mortality, that are estimated to affect 3–8% of the population [5,6]. AIDs can be characterized by an aberrant and chronic immune response. This aberrant immune response is induced following a breach of immunological tolerance to self. Environmental factors such as (viral) infections are suspected to play a causative role in this breach of tolerance. For instance, Epstein-Barr virus infection has been reported to be associated with multiple AIDs [7–9]. In the case of RA, evidence suggests that this does not occur via direct infection and escape of

Abbreviations: AIDs, autoimmune diseases; HLA, human leucocyte antigen; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; MS, multiple sclerosis; T1D, type 1 diabetes; CAAR, chimeric auto-antibody receptor; CAR, chimeric (auto)antigen receptor; SIGLEC, sialic acid binding Ig-like lectins; STALs, SIGLEC-engaging tolerance inducing antigenic liposomes; BCR, B cell receptor; ITAM, immunoreceptor tyrosine activation motif; ITIM, immunoreceptor tyrosine inhibition motif; PA, polyacrylamide; NP, nitrophenol; PIC, polyisocyanopeptide; ACPA, anti-citrullinated protein antibody; PLGA, poly-lactic-co-glycolic acid; ADC, (auto) antigen-drug conjugate; PR3, proteinase 3; GPA, granulomatosis with polyangiitis; ETA', truncated exotoxin A derived from Pseudomonas aeruginosa; EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; TTC, tetanus toxoid fragment C; CCP, cyclic citrullinated peptide; CNBz, carboxy-p-nitrobenzyl; ADEPT, antibody-directed enzyme prodrug therapy; FcγR, Fc-gamma receptor; HA, hyaluronic acid; ICAM-1, intracellular adhesion molecule 1; AChR, acetylcholine receptor; OVA, ovalbumin; BAR, B cell targeting antibody receptor; PV, pemphigus vulgaris; DSG, desmoglein; NSG, NOD-SCID-gamma; MuSK, muscle-specific tyrosine kinase; MG, myasthenia gravis; FVIII, factor VIII; FITC, fluorescein isothiocyanate; Tregs, T regulatory cells.

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autoreactive B cells [10], leaving other possible mechanisms such as molecular mimicry [11,12]. Similar mechanisms have also been proposed for the pathoetiology of MS [13].

Conventional treatments for AIDs often systemically suppress the immune system and can result in serious side effects such as severe infections. Therefore, a plethora of approaches is being investigated to achieve specific targeting and depletion of pathogenic, autoreactive cells. In this review, we will focus on ways to silence or deplete B cells in an antigen-specific manner as B cells are involved in multiple human AIDs. The latter is best exemplified by the success of B cell targeting therapies in several AIDs [14]. B cells can contribute to disease via several non-mutually exclusive mechanisms. For example, B cells can produce auto-antibodies that can directly bind to target tissue leading to its destruction or loss of function. Likewise, B cells can secrete many soluble mediators that can induce inflammation, recruit other immune cells or induce fibrosis. Lastly, B cells excel in the presentation of antigens to HLA class II-restricted T cells and thereby have the potential to steer and fuel autoreactive T cell responses [15]. We will discuss pathways that can be exploited to inhibit or deplete antigen-specific B cells, as well as the modalities that facilitate antigen-specific targeting such as immunomodulatory nanoparticles, (auto)antigen-drug conjugates (ADCs) and Chimeric Auto-Antibody Receptor (CAAR) or Chimeric (auto)Antigen Receptor (CAR) T cells.

2. The potential of SIGLEC-targeting

Lectins are carbohydrate-binding proteins. Many different lectins can be expressed by prokaryotic and eukaryotic cells. Their functions are diverse and include cell-adhesion, protein trafficking, protein degradation, endocytosis, phagocytosis and modulation of cell activity [16–19]. Relevant in the context of B cell targeting are sialic acid binding Ig-like lectins (SIGLECs), a subset of lectins [20]. SIGLECs have garnered considerable interest as drug targets in cancer and autoimmune disease, due to their predominant role in leukocytes as sialic acid binding cell-surface inhibitory or stimulatory receptors [21–23]. SIGLECs expressed on B cells are the inhibitory SIGLEC-2 (CD22) and SIGLEC-G/10 (murine and human orthologues, respectively). CD22 is an alpha 2–6 linked sialic acid binding SIGLEC, whereas SIGLEC-G/10 can bind both terminal alpha 2–3- and alpha 2–6 sialic acids [24,25]. CD22 has received the most attention as a potential drug target because of its B cell-restricted expression. Epratuzumab, a humanized anti-CD22 monoclonal antibody showed promising results in phase I/II trials of Non-Hodgkin's Lymphoma [26,27], Sjögren's Syndrome [28] and SLE [29,30]. Although Epratuzumab modulates B cell receptor signaling [31], it does not bind to its target in an antigen-directed manner and thus can elicit its immunomodulatory effects on the total CD22-expressing B cell population [32]. This means that Epratuzumab and other SIGLEC-targeted therapies might suffer from side effects associated with a broad targeting of B cell populations, similar to those observed for Rituximab [33–35]. Moreover, in two phase III clinical trials, Epratuzumab failed to meet its primary endpoint in the treatment of SLE [32]. In recent years, preclinical research into SIGLEC-targeted therapies has focused on antigen-directed, B cell-specific delivery by conjugating (auto-)antigens to immunomodulatory ligands that can interact with the relevant SIGLECs on various types of molecular scaffolds, such as SIGLEC-engaging tolerance inducing antigenic liposomes (STALs) or polymers. The concept of co-localizing (auto-)antigen and immunomodulatory ligands on scaffolds has resulted in a diverse array of versatile immunomodulatory platforms for delivery of antigen-targeted treatments, as we will discuss further below.

Mechanistically, the effects of targeting both CD22 and SIGLEC-G/10 are based on their respective roles as B cell receptor (BCR) complex inhibitory co-receptors [24,36]. The main function of the BCR complex is to transmit stimuli induced by (cognate) antigen recognition to downstream effector functions. Antigen-induced crosslinking of surface BCR will recruit various src-family phospho-tyrosine kinases resulting in phosphorylation of Ig α-β immunoreceptor tyrosine activation motif (ITAM) tyrosine residues. This will lead to the translocation of cytosolic protein tyrosine kinase Syk to the Ig α-β ITAM and its subsequent phosphorylation and activation of downstream pathways required for antibody production and proliferation [37]. Conversely, the BCR signaling threshold is tightly regulated by several B cell associated co-receptors. Upon ligation with the BCR complex, CD22 and SIGLEC-G/10 can inhibit the BCR-activation pathway and resulting effector functions. Inhibition via CD22 and SIGLEC-G/10 has been shown to be mediated through immunoreceptor tyrosine inhibition motifs (ITIM) located in their respective cytoplasmic tails and their ability to recruit protein tyrosine phosphatase SHP-1 [24,36]. Through its function as a phosphatase, SHP-1 can dephosphorylate components of the BCR pathway and counter BCR activation. Indeed, Ca^{2+}-flux inhibition induced by both CD22 and SIGLEC-G/10 has been shown to be SHP-1-mediated [24,38]. The inhibitory potential of CD22 and SIGLEC-G/10, combined with the ability to utilize the BCR specificity for (auto)antigen-targeted delivery, has spurred studies into the development of drug candidates. Early investigations into antigen-specific targeting of B cells via CD22 showed that polymers carrying multiple copies of the model antigen 2,4-dinitrophenyl (DNP) and terminal α2–6 linked sialic acids as CD22 ligands (CD22L) could co-ligate the BCR and CD22, facilitating an antigen-dependent manner to inhibit IgM DNP-specific B cells [39]. B cell inhibition was observed as evidenced by reduced phosphorylation of Syk and increased phosphorylation of CD22 in cells treated with the CD22L-carrying polymer, compared to cells treated with a control polymer lacking CD22 ligands. Moreover, almost complete abrogation of Ca^{2+}-signaling was seen in these B cells. Interestingly, no inhibition was observed in cells treated with polymers that carried DNP-antigen and CD22L on separate polymers, pointing towards the need for co-localization of both antigen and ligand on the same polymer [39]. A subsequent *in vivo* study where mice were immunized with polyacrylamide (PA) polymers functionalized with ~200 nitrophenol (NP) antigens and ~400 α2–6 sialosides displayed blunted or fully abrogated antibody response, depending on the affinity of sialoside ligands for murine CD22 and SIGLEC-G [40]. Intriguingly, this blunted response was also present during a re-challenge with NP, 30 days after initial immunization, which the authors interpreted as the (re-)establishment of humoral tolerance rather than temporary inhibition of the B cell response. Recently, in a study investigating the versatility of polyisocyanopeptide (PIC) polymers in the context of antigen-specific B cell phenotyping and modulation, many of these previous findings were recapitulated. PIC-polymers co-functionalized with autoantigen and CD22L resulted in inhibited Syk phosphorylation of *in vitro* stimulated B cells carrying an anti-citrullinated protein antibody (ACPA) BCR, the most prominent disease-specific autoreactive antibodies in RA. This inhibitory effect was not seen with PIC polymers containing antigen and control ligand and more importantly, antigen and CD22L functionalized on separate PICs also did not inhibit Syk phosphorylation, stressing the value of colocalization [41]. Next to polymers, SIGLEC-engaging tolerance-inducing antigenic liposomes have been employed to target SIGLECs in an antigen-directed manner. For instance, the inhibitory effects of STALS carrying T cell independent or T cell dependent B cell antigens and high affinity CD22L have been investigated on murine B cells *in vivo* [42]. For both T cell dependent and independent antigens, STALs reduced IgM and IgG production in response to an antigen challenge. Other indicators of B cell activation, such as Ca^{2+}-flux, CD86-expression, phosphorylation of BCR complex components, and cellular proliferation, were reduced. Additionally, STALs functionalized with high affinity SIGLEC-G-specific ligands were also shown to reduce Ca^{2+}-flux mediated in a SHP-1-mediated manner [38].

Taking antigen-specific CD22-targeting one step further, a preclinical study combining STALs with the immunoinhibitory drug Rapamycin exemplifies the potential benefits of drug synergism [43]. Antigen-displaying CD22L-carrying STALs and poly-lactic-co-glycolic acid (PLGA) nanoparticles containing Rapamycin, were co-administered to mice prone to develop arthritis. Mice treated with a combination of antigen-CD22-STALs and PLGA-Rapamycin displayed a lower autoantibody response that was additionally associated with a lower severity of arthritis. A 5-weekly dosed co-administration regiment of antigen-CD22-STALs and PLGA-Rapamycin delayed disease onset and reduced symptoms in mice with established disease. CD22-STALs have also been investigated for utility in inhibiting ACPA-expressing B cells [44]. The production of ACPA IgG and the differentiation of ACPA-expressing memory B cells to plasmablasts in RA patient cell cultures, were abrogated upon treatment with STALs. Additionally, it was reported that mice immunized with antigen-displaying CD22L-carrying STALs produced lower ACPA titers upon challenge with antigen, suggesting modulation of the B cell response to citrullinated antigens.

3. Delivery of drug and/or inhibitory signals

The unique ability of B cells to bind and internalize cognate antigens can not only be used to engage SIGLECs, but also to enable antigen-specific delivery of effector molecules such as drugs and antibodies that engage other (inhibitory) cell surface receptors. This yields a category of antigen-specific B cell targeting modalities that may directly prompt inhibition or depletion through *e.g.* induction of apoptosis/cell lysis.

3.1. Antigen-drug conjugates

Conjugating (auto)antigens to drugs or toxins shows promise as an approach to eliminate autoreactive B cells. In principle, an ADC will specifically bind to and be internalized by the autoreactive BCR expressed by B cells, leaving the gross majority of the B cell compartment unaffected. Such biologicals have the advantage of being relatively small, thus aiding manufacturability. An example of this is a conjugate containing inactive antigen proteinase 3 (PR3) and human angiogenin toxin to target anti-PR3-specific B cells that was studied in the treatment of granulomatosis with polyangiitis (GPA) two decades ago [45]. PR3 is a serine protease residing in neutrophil granules which have also been reported to be relocated to the cell membrane in certain conditions. Anti-PR3 antibodies play a pathogenic role in GPA by binding to neutrophils, thereby causing their activation in blood vessels and leading to subsequent vasculitis-induced lesions [46]. The rPR3-angiogenin fusion protein induced apoptosis in PR3-specific hybridoma cell lines while leaving control cell lines intact, showing the promise of antigen-drug conjugates to treat autoimmune disease [45]. We do note that no follow-up studies based on this concept have been published since this first report.

Truncated exotoxin A derived from Pseudomonas aeruginosa (ETA') is another potent toxin used in fusion proteins. In the experimental autoimmune encephalomyelitis (EAE) mouse model for MS, anti-MOG antibodies mediate pathogenic demyelination. A conjugate containing the extracellular domain of myelin oligodendrocyte glycoprotein (MOG) linked to ETA' was developed and tested in this model [47]. The MOG-ETA' fusion immunotoxin was shown to specifically target and deplete MOG-reactive hybridoma cells *in vitro* as well as primary MOG-reactive B cells isolated from MOG-specific Ig heavy-chain knock-in mice (IgH MOG) [47]. Similarly, a fusion protein comprising ETA' and tetanus toxoid fragment C (TTC) specifically binds to and targets TTC-reactive hybridoma cells, as well as primary B cells from immunized donors [48]. TTC-ETA' decreased the TTC-reactive IgG producing cells in comparison to the TTC protein without a toxic domain [48]. While not directly reporting effects on B cells, in another study that employed an EAE model using ADCs consisting of the EAE-specific antigen PLP139–151 linked to dexamethasone, mice were more potently protected from the development of symptoms than mice that receiving dexamethasone treatment alone [49].

Though more studies are needed to gain further insight in ADCs effects, current literature demonstrates the potential of ADCs to silence autoimmunity through antigen-specific depletion of autoreactive B cells. However, several potential therapeutic challenges remain. Firstly, the binding of BCR to cognate antigen may activate, rather than inhibit, the B cell. Secondly, ADCs will encounter autoantibodies present in the body which can neutralize functional ADCs by binding and blocking their activity. Although this effect can be circumvented by increased dosing or plasmapheresis, other possibilities circumventing the presence of neutralizing antibodies are also explored. For example, Lelieveldt et al. demonstrated the absence of cyclic citrullinated peptide (CCP) binding to ACPA-expressing B cells after addition of a carboxy-p-nitrobenzyl (CNBz) blocking group to the CCP-antigen [50]. After enzymatic removal by nitroreductase, full restoration of antigen-binding to ACPA-expressing B cells was achieved. Additionally, CCP(CNBz) linked to the cytotoxic ribosome inhibitor Saporin only induced ACPA-expressing B cell specific cell death in the presence of nitroreductase [50]. While *in vivo* data of such targeted delivery and activation is still lacking, this technique might allow antigen-specific elimination of autoreactive B cells while shielding the compound from circulating autoantibodies. The latter is accomplished by embedding this technique within the ADEPT-approach (antibody-directed enzyme prodrug therapy), where an enzyme-labeled antibody is administered first. After subsequent administration of the antigen-drug conjugates, the conjugates will become activated only in proximity of the target cell [51]. Future studies are required to thoroughly assess the preventive and therapeutic potential of ADCs in the context of autoimmunity, although the clinical applicability of two-step approaches such as ADEPT is likely limited by the need to manufacture and study multiple combined products at clinical grade.

3.2. Antibodies and antigen-Fc conjugates

A less common modality for targeting autoreactive immune cells are autoantigen-directed monoclonal antibodies. On the one hand, these antibodies have been shown to directly exacerbate inflammation by binding to their respective cognate autoantigen. On the other hand, data suggests benefits in specifically targeting autoreactive B cells. A monoclonal antibody directed against insulin (mab123) has been evaluated in NOD mice. Mab123 recognized and eliminated insulin-reactive B cells when endogenous insulin was bound to the autoreactive BCR. Importantly, mab123 did not bind insulin when associated with the insulin receptor, making the accumulation of antibody-insulin complexes and the subsequent potentially pathogenic downstream effects unlikely [52]. The mode of action of insulin-specific B cell reduction was not investigated but it is conceivable that it involves Fc-gamma receptor II (FcγRII) by linking the BCR to FcγRII via the Fc-domain of the autoantigen-specific antibody. Another way of benefiting from such Fc-mediated targeting mechanisms is being explored by Akston Biosciences. They aim to deplete insulin-reactive B cells by using an Fc-insulin conjugate named AKS-107. Although still unpublished, investigational new drug (IND) applications state the ability of AKS-107 to prevent T1D in mouse models and its safety in non-human primates [53]. A report on the canine variant AKS-218d showed comparable glycemic control, clinical signs & bodyweight using this once-weekly injection compared to twice-daily insulin shots the dogs received before that in 4 out of 5, with the fifth developing anti-drug antibodies [54].

3.3. Nanoparticles

Drug-antigen-carrying nanoparticles can be used to target antigen-specific cells. These nanoparticles can be used to deliver immunosuppressive drugs in an antigen-specific manner to silence B cells, through various modalities, such as encapsulation or ligation. For example, it was reported in multiple murine disease models that synthetic antigen-expressing vesicles containing encapsulated rapamycin, an inhibitory immunomodulator, were able to inhibit cellular and humoral immune responses to immunogenic challenges [55]. Free rapamycin combined with either free or encapsulated antigen did not inhibit the antigen-specific immune response. Intriguingly, the data showed that the inhibition of the humoral immune response to immunogenic rechallenges induced by these vesicles lasted for more than 200 days and was hypothesized to be mediated by the antigen-specific induction of $CD4^+$ $FoxP3^+$ T regulatory cells (Tregs).

Polymer-based nanoparticles on the other hand, do not carry encapsulated drugs, but rather carry the drug or effector molecule on the polymer backbone. An example of these are hyaluronic acid (HA) polymers carrying an encephalitogenic peptide as well as a peptide that inhibits intracellular adhesion molecule 1 (ICAM-1) [56–58]. Using an EAE murine model, it was reported that polymers carrying both autoantigen and inhibitory peptide reduce disease severity and delay disease. Likewise, induction of B cell anergy by inducing sustained BCR engagement ultimately blunted Ca^{2+}-flux after IgM stimulation [59].

3.4. Plasma cell targeting

Current therapies used for B cell targeting, such as Rituximab (anti-CD20) and Epratuzumab (anti-CD22) do not affect plasma cell numbers due to the lack of expression of the respective target proteins on plasma cells. Plasma cells can thus continue to produce autoreactive antibodies in patients undergoing conventional B cell depletion therapy. Therefore, development of therapies focused on depleting plasma cells in an antigen-specific manner, are highly valuable in case disease is primarily driven by pathogenic autoantibodies produced by long-lived plasma cells. As surface immunoglobulins are considered to be downregulated on plasma cells, targeting autoreactive plasma cell clones in an antigen-specific setting is more complex than targeting B cells. Nonetheless, also the plasma cell compartment can be targeted antigen-specifically, for example by using “affinity matrices” of anti-CD138 and anti-CD44 F(ab)$_2$-fragments conjugated to the antigen of interest. The F(ab)$_2$-fragments bind to the plasma cell surface CD44 and CD138 molecules and are able to bind secreted immunoglobulins with the antigen-fragment of the conjugate (46). Complement activation induced by the immunoglobulins bound to these receptors can subsequently facilitate cell lysis. Using plasma cells from an established murine model of autoimmune myasthenia gravis in *ex vivo* experiments, the efficacy of this approach to deplete acetylcholine receptor (AChR)-specific plasma cells, while sparing the non-specific plasma cells, was shown. In a 2020 follow up study from the same research group, the utility of this approach was reported *in vivo* [60]. More specifically, mice immunized with ovalbumin (OVA) that subsequently received an injection of an OVA-anti-CD138-conjugate (OVA-C) showed a drop in OVA-specific plasma cells in the bone marrow plasma cell population that was not seen in control chicken gamma globulin-specific plasma cells. Moreover, this was associated with a reduction in OVA-antibody titers in treated mice. Thus, these results indicate the antigen-specific depletion of plasma cells from the bone marrow of mice, providing an option for the treatment of antibody-mediated AIDs that do not respond to (anti-CD20 or CD22-mediated) B cell depletion.

4. Cell therapies

Despite the challenges that need to be overcome to reach the clinic [61], CAR-T cells have now shown great potential as anticancer therapy [62]. In general, CAR-T cells express a CAR consisting of an intracellular signaling domain, often derived from CD3ζ and two co-stimulatory domains derived from *e.g.* CD28 and CD137 (4–1BB) [63]. The intracellular domain induces T cell activation upon antigen binding by the extracellular domain containing monoclonal antibody single-chain variable fragments [64]. This also underlines a major advantage of CAR-T cells: they recognize integral proteins expressed on target cells instead of antigenic peptides presented in the context of MHC-I or MHC-II. When effector T cells are transduced with a chimeric receptor, the antigen-induced T cell activation will typically lead to the eradication of the target cell. Given their antigen-specific recognition and cytolytic abilities, CAR-T cells have potential in treatment of AIDs. Recently, CD19-directed CAR-T cells were reported to induce clinical and serologic remission in a patient suffering from severe and refractory SLE [65]. Remarkably, CAR-T cell related adverse events such as cytokine release syndrome were not observed in this patient. In line with this observation, a recent article described only mild cytokine release syndrome after effective treatments of five SLE patients with CD19-directed CAR-T cells [66]. We hypothesize this to be due to a lower target antigen load in comparison to *e.g.* B cell malignancies [67], indicating CAR-T therapies in AIDs may induce less adverse effects.

4.1. CAR- and CAAR-T effector cells

Currently approved CAR-T effector cells target general expression markers and, in doing so, also eliminate non-pathogenic cells. For a general review of the use of CAR-T cells for treatment of AIDs, we suggest Orvain et al. [68]. For treatment of AIDs mediated by autoantibody-producing B cells however, specific targeting of the autoreactive BCR of pathogenic B cells in an antigen-specific manner is desirable. In CAAR-T cells, also known as B cell targeting antibody receptor T (BAR-T) cells, the conventional CAR concept is turned around. CAAR-T cells are constructed to express a specific (auto)antigen as the extracellular binding domain. By binding to BCRs expressed by autoreactive B cells, antigen-specific binding and cell death is elicited. Through this approach, autoreactive B cells specific for both intracellular and extracellular antigens can be targeted whereas regular CAR-T cell therapy is restricted to extracellular antigens. This concept was described in a study published by Ellebrecht et al., where the authors demonstrate the potential of CAAR-T cell treatment for pemphigus vulgaris (PV) [69]. PV is an autoantibody-mediated autoimmune disease in which desmoglein (DSG) 3 is considered the primary autoantigen [70]. CAAR-T cells containing DSG3 as “T cell recognition domain”, linked to CD137-CD3ζ signaling domains, specifically eliminated anti-DSG3 BCR expressing hybridoma cells *in vitro* and showed sustained cytotoxicity, even in the presence of soluble anti-DSG3 antibodies [69]. Although only having a short-term follow-up, *in vivo* efficacy of DSG3-CAAR-T cells was demonstrated using NOD-SCID-gamma (NSG) mice injected with DSG3-BCR expressing hybridomas followed by DSG3-CAAR-T injection on day 5. On day 14, anti-DSG3 antibody levels were reduced and oral blistering as well as autoantibody binding vanished and hybridoma outgrowth were delayed [69]. Furthermore, DSG3-CAAR-T cells did not show off-target cytotoxicity to $CD64^+$ ($Fc\gamma R^+$) K562 cells *in vitro* or $Fc\gamma R^+$-expressing cells (*e.g.* monocytes) *in vivo* [69]. Additional pre-clinical data showed the specific killing capacity of DSG3-CAAR-T cells against primary B cells expressing anti-DSG3 IgG isolated from patients with PV [71]. This supported the first in-human trial investigating the potential of CAAR-T cells to treat autoimmunity (NCT04422912). Similarly, the same group recently reported positive effects of CAAR-T cells expressing muscle-specific tyrosine kinase (MuSK) to target anti-MuSK B cells in the context of MuSK myasthenia gravis (MG) [72]. These CAAR-T cells are currently also being investigated in a phase 1 clinical study (NCT05451212).

Likewise, CAAR-T cells expressing the immunodominant factor VIII (FVIII) domains as autoantibody receptor are explored to treat hemophilia patients that have developed anti-FVIII antibodies to therapeutic FVIII [73]. Using these FVIII-specific CAAR-T cells, the specific elimination of FVIII-BCR expressing hybridoma cells *in vitro* and *in vivo* was shown. Additionally, adoptive transfer of FVIII-CAAR-T cells into hemophilic mice significantly lowered anti-FVIII antibody production [73], thereby supporting the potential of CAAR-T cells in treating detrimental anti-drug responses.

The CAAR-T approaches discussed so far have in common that they exclusively target one antigen, whereas in several AIDs, multiple

Table 1
Overview of described modalities used to antigen-specifically inhibit, deplete or silence autoreactive B cells.

Modality	Primary target	(Most studied) model antigen	Carrying	Inhibiting	Depleting & silencing	Concept history	Current status in AI context
Monoclonal antibody	BCR	Insulin	/	✓	✓	First monoclonal produced in 1973 [96]. In 1986, Muromonab-CD3 (anti-CD3), used for treatment of graft rejection in transplantation, became the first FDA-approved monoclonal antibody therapy.	Several mAbs are used for treatment of autoimmune disease (e.g. Rituximab (anti-CD20) and Epratuzumab (anti-CD22)) but not antigen-specifically. In antigen-specific context, AKS-107 was reported to lead to a reduction in insulin-specific B cells in T1D mouse and NHP models.
Polymers	BCR	MS autoantigen peptide PLP	Various, e.g. CD22	✓	✓	Concept of polymers [97].	Reduced disease severity and delayed symptoms onset in EAE mouse model [98].
Vesicular particles (STALs, nanoparticles)	BCR, other APCs	OVA or OVA peptides	Ligands for cellular receptors (e.g. SIGLEC-G, CD22) or drugs, e.g. rapamycin	✓	✓	Concept of nanoparticles [99]	Tolerogenic in mouse models [100], decrease in ACPA IgG RA mouse models and patient cell cultures [44].
Antigen-drug conjugates	BCR	MS autoantigen MOG	Various, including toxins and immunosuppressants	✓	✓	First successful ADC clinical trial in 1983 [101], Mylotarg FDA-approved for acute myeloid leukemia in 2000 (although it received a black-box warning only 1 year later and was eventually relicensed at a lower dose) [102].	Antigen-dexamethasone conjugate: EAE murine MS model protected from symptom onset [49].
$F(ab)_2$ fragments	CD138 & CD44 on plasma cells	Various	/		✓	See mAbs above. Abciximab (anti-glycoprotein IIb/IIIa) for clot prevention was the first FDA-approved $F(ab)_2$ therapy in 1994 [103].	Reduction in antibody titers and number of antigen-specific B cells in mice [60].
CAR-T effector cells	FITC	FITC-labeled autoantigenic peptides	/		✓	First CAR-T cells engineered in 1989-1993 [104,105], first clinical application in humans in the context of leukemia in 2009 [106].	*In vitro* killing of murine immunization-derived hybridoma cells and autoreactive B cells from RA patients [74].
CAAR-T regulatory cells	BCR	Insulin	/		✓	See above, first mouse model with CAR-T regs published in 2016 [107].	*In vitro* stable and functional insulin-specific CAAR-Tregs did not prevent spontaneous diabetes development in NOD/Ltj mice [84].
CAAR-T/BAR-T cells	BCR	DSG	/		✓	See above, first paper describing an engineered CAAR in 2016 [69].	Ongoing Phase I clinical trial in PV and MuSK MG patients (NCT04422912, NCT05451212).

ACPA: anti-citrullinated protein antibodies, BAR-T cell: B cell targeting antibody receptor T cell, BCR: B cell receptor, CAAR-T cell: chimeric autoantigen receptor T cell, CAR-T cell: chimeric antigen receptor T cell, CD138: Syndecan-1, transmembrane heparan sulfate proteoglycan expressed by plasma cells, CD44: cell surface glycoprotein, DSG: desmoglein, primary autoantigen for pemphigus vulgaris, EAE: experimental autoimmune encephalomyelitis, FITC: fluorescein isothiocyanate, FVIII: immunodominant factor VIII, ICAM-1: intercellular adhesion molecule 1, LABL: ICAM-1 inhibitor peptide derived from leukocyte function associated antigen-1, MS: multiple sclerosis, NHP: non-human primate, PLGA: poly-lactic-co-glycolic acid, PV: pemphigus vulgaris, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, STAL: SIGLEC-engaging tolerance-inducing antigenic liposomes.

autoantigens are involved. Targeting multiple autoreactive B cell populations simultaneously would be ideal in these diseases and which could potentially be addressed by combining multiple CAAR-T cells expressing different (auto)antigens. This could be achieved by generating a CAAR construct that allows the coupling of different antigens. First studies have demonstrated the technical feasibility of such approaches by generating “conventional” CAR-T cells expressing an anti-fluorescein isothiocyanate (FITC) receptor [74]. By combining various FITC-labeled autoantigenic peptides, this single anti-FITC CAAR-T cell can target multiple autoreactive B cell populations. Indeed, specific killing of autoreactive-BCR expressing hybridoma cells as well as primary ACPA-expressing B cells from patients with RA has been shown by CAAR-T cells generated in this manner [74]. Whether this approach will work out *in vivo* remains to be determined, but it is likely that this will not involve an anti-FITC CAAR-T cell as FITC-labeled antigens are expected to be immunogenic *in vivo* [75]. However, other CAAR-T cells targeting less immunogenic groups that can be coupled to antigens might offer promise for the generation of multiple CAAR-T cells and/or CAAR-T cells targeting post-translational modifications such as citrullinated proteins.

4.2. CAAR-T regulatory cells

Exogenously expanded T regulatory cells (Tregs) have successfully demonstrated their suppressive abilities in the context of several AIDs in mice [76–78]. Preclinical studies have shown the efficacy of antigen-specific Tregs over polyclonal Tregs in controlling AIDs through mediating tolerance [79–81]. Additionally, the risk of generalized immunosuppression is reduced because Tregs are expected to localize predominately at the site of antigen. However, self-antigen-specific Tregs are extremely rare and have, to our knowledge, not yet been successfully expanded *ex vivo*. Therefore, genetic modification to design Tregs specific for relevant antigens is desired to induce tolerance. Most CAAR-Tregs tested in context of AIDs focused on restoring general

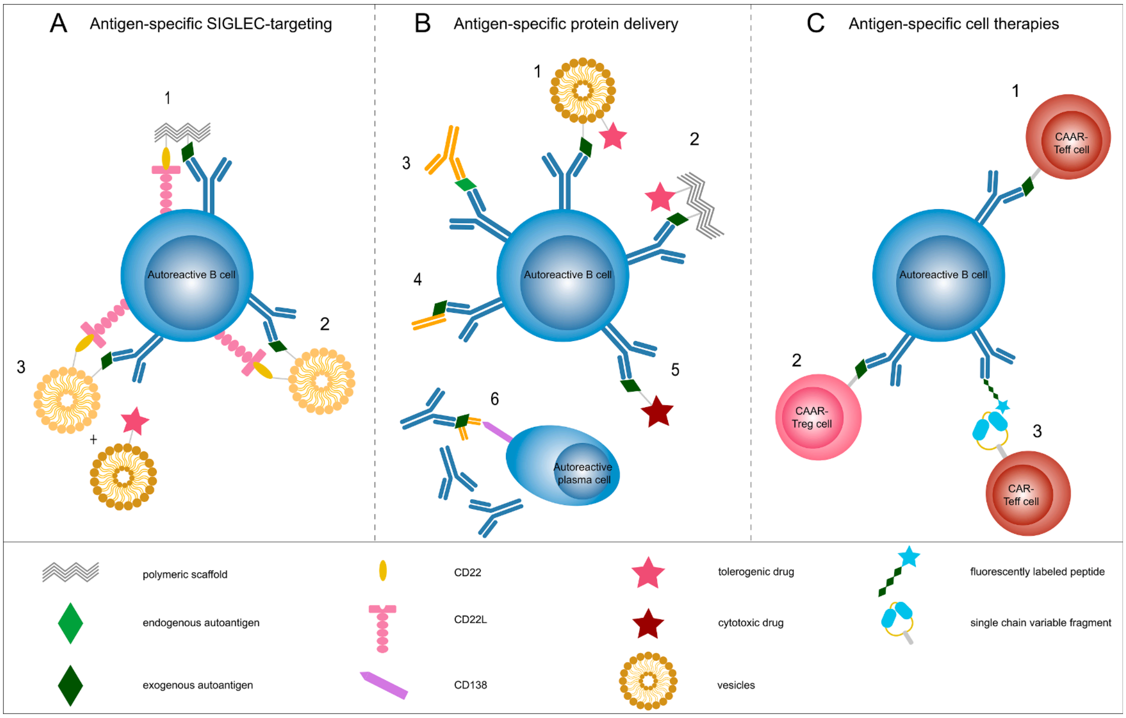

Fig. 1. Approaches to target autoreactive B cells in an antigen-dependent manner cells.
A. Antigen-specific SIGLEC-targeting: (1) polymeric scaffolds containing antigen and CD22L; (2) STALs expressing antigen and CD22L; (3) co-administration of STALs containing CD22L and antigen with PLGA vesicles containing silencing drugs. B. Antigen-specific protein delivery: (1) vesicles delivering silencing drugs; (2) polymeric scaffolds delivering silencing drugs; (3) monoclonal antibodies binding antigen bound to BCRs; (4) Fc-fusion proteins targeting autoreactive BCRs; (5) antigen-drug conjugates delivering cytotoxic drugs; (6) autoreactive plasma cell targeting by antigen-anti-CD138 F(ab)$_2$ conjugates. C. Antigen-specific cell therapies: (1) CAAR-T effector cells expressing autoantigens; (2) CAAR-T regulatory cells expressing autoantigens; (3) CAR-T effector cells expressing scFv reactive to a single 'tag' recombinantly linked to (various) autoantigen(s).

immunotolerance rather than specifically silencing autoreactive B cells [82–85]. While the studies referenced here show promising results in reducing disease burden in mice for various B cell-mediated AIDs, the reported data do not assess the therapeutic effect on these B cells specifically and therefore fall outside of the scope of this review.

CAAR-Tregs targeting autoreactive B cells have been studied in the setting of unwanted B cell immunity against FVIII [86]. The antibody response of hemophilic patients treated with therapeutic FVIII hinders the efficacy of FVIII treatment and inhibiting the FVIII-specific B cell response is desired. FVIII specific CAAR-Tregs have been shown to be able to inhibit the anti-FVIII antibody response of FVIII-immunized mice. The FVIII-specific CAAR-Tregs suppress FVIII-specific memory B cells and the development of anti-FVIII antibody secreting cells, even in the presence of antibodies against FVIII [87].

A potential risk of CAAR-Tregs could come from the instable nature of FoxP3. Inflammatory environments might cause FoxP3 down-regulation, causing CAAR-Tregs to switch to a CAAR-T effector phenotype and thereby exacerbate inflammation [88]. Several strategies have been explored to avoid this, such as the introduction of suicide switches that are activated upon FoxP3 inactivation [89,90]. Although encouraging progress has been made, mechanisms of the CAAR-Treg approach should be investigated in more detail to diminish safety concerns.

5. Conclusion

The mechanisms underlying the breach of immunological tolerance to self and the pathogenesis of autoimmune disease remain largely unknown. The HLA locus has been shown to be the predominant genetic risk factor for most AIDs, with more modest and disease-specific contributions to AIDs from miscellaneous genetic and environmental risk factors [91]. Lack of knowledge on the causative factors in the breach of tolerance complicates the development of treatments. However, despite incomplete knowledge on the etiology of AIDs, treatments have improved considerably over time. In this review, we have discussed various emerging modalities that are focused on antigen-specific inhibition, depletion or silencing of B- and plasma cell compartments, with the aim of mitigating the primary B cell effector functions and their subsequent immunopathologies. Several of the discussed modalities seem promising *in vitro* and *in vivo*, though their impact in the context of human clinical trials remains uncertain (see Table 1 for an overview of the included modalities, their history and current state of development and Fig. 1 for a graphical summary). Ideally, novel therapies would be curative. However, this is a high bar to meet for many treatments and 'solely' treating symptomatic disease while keeping side effects low would, potentially, already greatly benefit patients. It seems that strategies such as CD22-targeting and the use of Rapamycin-containing vesicles can induce antigen-specific B cell silencing, but require maintenance of therapy. Depleting therapies, mediated by *e.g.* CAR-T cells, have shown remarkable curative potential in the clinic but have lacked antigen-specificity. Nevertheless, it is tempting to speculate on the curative capacity of antigen-specific CAAR-T cells by depleting pathogenic B cells and restoring immunological tolerance. This can also be accomplished by antigen-specific delivery of cytotoxic drugs, a concept benefiting from high versatility in terms of (molecular) properties of the delivery platform, drug types and combinations thereof. Although in this review, we suggest that antigen-specific targeting of autoreactive B cells can result in overall improved treatments and has potential to reduce treatment side-effects, these strategies come with an inherent limitation. Namely, that the disease-specific autoantigen(s) or surrogate antigens must be known. For many common AIDs (some of) the autoantigens are defined [92], although in other AIDs that are characterized by multiple autoantibody responses, the relative contributions to the overall disease is not well understood. Thus, it may not be easy to pinpoint the antigens that need to be targeted in order to achieve clinical benefit.

To conclude, adapting existing therapeutic modalities to target autoreactive B cells in an antigen-specific manner is desired to ultimately come to improved and potential curative treatments for AIDs with minimal impact on the non-autoimmune compartment. Given the heterogeneity of AIDs, investigations on the curative potential of these platforms and compounds could be a promising road to follow. Especially the versatile and promising routes explored to generate T cells expressing a recombinant receptor directly recognizing autoreactive B cells could represent a way to permanently eradicate pathogenic B cell responses and thereby potentially create novel means to induce long term or even permanent remission of disease activity.

6. Box 1

While the approaches discussed in this review all target the (pathogenic) autoreactive B cell response, another strategy that we did not include here is to modulate the associated T cell response. This approach requires knowledge of the primary target antigen(s), which for several AIDs is unknown. While multiple studies on tolerizing vaccines show amelioration of disease and a decline in antigen-specific antibodies, direct effects on B cells have scarcely been reported and therefore we have not specifically included this aspect in this overview which is focussing on antigen-specific B cell targeting. Nonetheless, multiple applications have shown successful results *in vitro* and *in vivo* in pre-clinical animal models, also in presumed B cell mediated disease. One recent study to highlight involves an autoantigen encoded mRNA liposomal formulation that delivers m1ψ-modified mRNA to lymphoid $CD11^{+}$ APCs in a non-inflammatory context [93]. In the EAE mouse model, this tolerogenic vaccination induces a large and active T_{reg} population that directly and indirectly (via bystander activation) prevents and reverts EAE. While the durability of these and similar study results is unknown, the application of mRNA vaccines throughout the SARS-CoV-2 pandemic has shown that these can easily and cheaply be produced and are safe to use even in patients with autoimmune disease [94]. For an extensive review about tolerogenic vaccines used for the induction of antigen-specific tolerance describing the different platforms -DNA, RNA, protein & peptide- as well as the prominent mediating cell types in a range of AIDs, see Moorman, Sohn & Phee [95].

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Declaration of Competing Interest

We declare no conflict of interest.

References

[1] T. Kamradt, N.A. Mitchison, Advances in immunology: tolerance and autoimmunity, N. Engl. J. Med. 344 (9) (2001) 655–664.
[2] L. Van Parijs, A.K. Abbas, Homeostasis and self-tolerance in the immune system: turning lymphocytes off, Science 280 (1998) 243–248.
[3] L. Wang, F.S. Wang, M.E. Gershwin, Human autoimmune diseases: a comprehensive update, J. Intern. Med. 278 (4) (2015), 369-95.
[4] L. Moroni, I. Bianchi, A. Lleo, Geoepidemiology, gender and autoimmune disease, Autoimmun Rev. 11 (6–7) (2012). A386-92.
[5] G.S. Cooper, B.C. Stroehla, The epidemiology of autoimmune diseases, Autoimmun. Rev. 2 (3) (2003) 119–125.
[6] G.S. Cooper, M.L. Bynum, E.C. Somers, Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases, J. Autoimmun. 33 (3–4) (2009) 197–207.
[7] N. Balandraud, J. Roudier, Epstein-Barr virus and rheumatoid arthritis, Joint Bone Spine 85 (2) (2018) 165–170.
[8] K. Bjornevik, et al., Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis, Science 375 (2022) 296–301.
[9] N.R. Jog, J.A. James, Epstein Barr virus and autoimmune responses in systemic lupus erythematosus, Front. Immunol. 11 (2020), 623944.
[10] S. Kroos, et al., Absence of Epstein-Barr virus DNA in anti-citrullinated protein antibody-expressing B cells of patients with rheumatoid arthritis, Arthritis Res. Ther. 24 (1) (2022) 230.
[11] I. Fanelli, et al., Reactivity of rheumatoid arthritis-associated citrulline-dependent antibodies to Epstein-Barr virus nuclear antigen1-3, Antibodies (Basel) 11 (1) (2022).
[12] M. Cornillet, et al., In ACPA-positive RA patients, antibodies to EBNA35-58Cit, a citrullinated peptide from the Epstein-Barr nuclear antigen-1, strongly cross-react with the peptide beta60-74Cit which bears the immunodominant epitope of citrullinated fibrin, Immunol. Res. 61 (1–2) (2015) 117–125.
[13] T.V. Lanz, et al., Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM, Nature 603 (7900) (2022) 321–327.
[14] J.L. Barnas, R.J. Looney, J.H. Anolik, B cell targeted therapies in autoimmune disease, Curr. Opin. Immunol. 61 (2019) 92–99.
[15] T. Dorner, A.M. Jacobi, P.E. Lipsky, B cells in autoimmunity, Arthritis. Res. Ther. 11 (5) (2009) 247.
[16] K. Drickamer, M.E. Taylor, Biology of animal lectins, Annu. Rev. Cell Biol. 9 (1993) 237–264.
[17] Y.C. Lee, Biochemistry of carbohydrate-protein interaction, Faseb J. 6 (13) (1992) 3193–3200.
[18] D. Fiete, et al., A hepatic reticuloendothelial cell-receptor specific for So4-4 gainac-Beta-1,4 glcnac-Beta-1,2man-Alpha that mediates rapid clearance of lutropin, Cell 67 (6) (1991) 1103–1110.
[19] N. Razi, A. Varki, Masking and unmasking of the sialic acid-binding lectin activity of CD22 (Siglec-2) on B lymphocytes, in: Proceedings of the National Academy of Sciences of the United States of America 95, 1998, pp. 7469–7474.
[20] L.D. Powell, A. Varki, I-TypeLectins, J. Biol. Chem. 270 (24) (1995) 14243–14246.
[21] C.D. Rillahan, et al., Disubstituted sialic acid ligands targeting siglecs CD33 and CD22 associated with myeloid leukaemias and B cell lymphomas, Chem. Sci. 5 (6) (2014) 2398–2406.
[22] J. Carnahan, et al., Epratuzumab, a CD22-targeting recombinant humanized antibody with a different mode of action from rituximab, Mol. Immunol. 44 (6) (2007) 1331–1341.
[23] C. Bull, et al., Sialic acid mimetics to target the sialic acid-siglec axis, Trends Biochem. Sci. 41 (6) (2016) 519–531.
[24] L. Nitschke, et al., CD22 is a negative regulator of B-cell receptor signalling, Current Biol. 7 (2) (1997) 133–143.
[25] B.H. Duong, et al., Decoration of T-independent antigen with ligands for CD22 and Siglec-G can suppress immunity and induce B cell tolerance in vivo, J. Exp. Med. 207 (1) (2010), 173-87.
[26] J.P. Leonard, et al., Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma, J. Clin. Oncol. 21 (16) (2003) 3051–3059.
[27] J.P. Leonard, et al., Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: phase I/II clinical trial results, Clin. Cancer Res. 10 (16) (2004) 5327–5334.
[28] S.D. Steinfeld, et al., Epratuzumab (humanised anti-CD22 antibody) in primary Sjogren's syndrome: an open-label phase I/II study, Arthritis Res. Ther. 8 (4) (2006).
[29] T. Dorner, et al., Initial clinical trial of epratuzumab (humanized anti-CD22 antibody) for immunotherapy of systemic lupus erythematosus, Arthritis Res. Ther. 8 (3) (2006).
[30] D.J. Wallace, et al., Efficacy and safety of epratuzumab in patients with moderate/severe active systemic lupus erythematosus: results from EMBLEM, a phase IIb, randomised, double-blind, placebo-controlled, multicentre study, Ann. Rheum. Dis. 73 (1) (2014) 183–190.
[31] T. Dorner, et al., The mechanistic impact of CD22 engagement with epratuzumab on B cell function: implications for the treatment of systemic lupus erythematosus, Autoimmun. Rev. 14 (12) (2015), 1079-86.
[32] M.E. Clowse, et al., Efficacy and safety of epratuzumab in moderately to severely active systemic lupus erythematosus: results from two phase III randomized, double-blind, placebo-controlled trials, Arthritis Rheumatol. 69 (2) (2017) 362–375.
[33] R.F. van Vollenhoven, et al., Long-term safety of rituximab in rheumatoid arthritis: 9.5-year follow-up of the global clinical trial programme with a focus on adverse events of interest in RA patients, Ann. Rheum. Dis. 72 (9) (2013), 1496-502.
[34] D. Nicholls, et al., A retrospective chart review of the use of rituximab for the treatment of rheumatoid arthritis in Australian rheumatology practice, Int. J. Rheum. Dis. 17 (2014) 755–761.
[35] M.F. Doran, et al., Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study, Arthritis Rheum. 46 (9) (2002), 2287-93.
[36] G. Whitney, S. Wang, H. Chang, K. Cheng, P. Lu, X.D. Zhou, W. Yang, M. McKinnon, M. Longphre, A new siglec family member, siglec-10, is expressed in cells of the immune system and has signaling properties similar to CD33, Eur. J. Biochem. 268 (2001) 6083–6096.
[37] J.M. Dal Porto, et al., B cell antigen receptor signaling 101, Mol. Immunol. 41 (6–7) (2004) 599–613.
[38] F. Pfrengle, et al., Copresentation of antigen and ligands of Siglec-G induces B cell tolerance independent of CD22, J. Immunol. 191 (4) (2013), 1724-31.
[39] A.H. Courtney, et al., Sialylated multivalent antigens engage CD22 in trans and inhibit B cell activation, Proc. Natl. Acad. Sci. U S A 106 (8) (2009), 2500-5.

[40] B.H. Duong, et al., Decoration of T-independent antigen with ligands for CD22 and Siglec-G can suppress immunity and induce B cell tolerance in vivo, J. Experimental Med. 207 (1) (2010) 173–187.
[41] H. Kristyanto, et al., Multifunctional, multivalent PIC polymer scaffolds for targeting antigen-specific, autoreactive B cells, ACS Biomater. Sci. Eng. 8 (4) (2022) 1486–1493.
[42] M.S. Macauley, et al., Antigenic liposomes displaying CD22 ligands induce antigen-specific B cell apoptosis, J. Clin. Invest 123 (7) (2013), 3074-83.
[43] A. Srivastava, et al., Tolerogenic nanoparticles impacting B and T lymphocyte responses delay autoimmune arthritis in K/BxN mice, ACS Chem. Biol. (2021).
[44] K.J. Bednar, et al., Exploiting CD22 to selectively tolerize autoantibody producing B-cells in rheumatoid arthritis, ACS Chem. Biol. 14 (4) (2019) 644–654.
[45] K.S. Reiners, et al., Selective killing of B-cell hybridomas targeting proteinase 3, Wegener's autoantigen, Immunology 112 (2) (2004), 228-36.
[46] R. Kettritz, How anti-neutrophil cytoplasmic autoantibodies activate neutrophils, Clin. Exp. Immunol. 169 (3) (2012), 220-8.
[47] T. Nachreiner, et al., Depletion of autoreactive B-lymphocytes by a recombinant myelin oligodendrocyte glycoprotein-based immunotoxin, J. Neuroimmunol. 195 (1–2) (2008) 28–35.
[48] D. Klose, et al., Novel fusion proteins for the antigen-specific staining and elimination of B cell receptor-positive cell populations demonstrated by a tetanus toxoid fragment C (TTC) model antigen, BMC Biotechnol. 16 (2016) 18.
[49] C.J. Pickens, et al., Antigen-drug conjugates as a novel therapeutic class for the treatment of antigen-specific autoimmune disorders, Mol. Pharm. 16 (6) (2019) 2452–2461.
[50] L.P.W.M. Lelieveldt, et al., Sequential prodrug strategy to target and eliminate ACPA-selective autoreactive B cells, Mol. Pharm. 15 (12) (2018) 5565–5573.
[51] S.K. Sharma, K.D. Bagshawe, Antibody directed enzyme prodrug therapy (ADEPT): trials and tribulations, Adv. Drug Deliv. Rev. 118 (2017) 2–7.
[52] R.A. Henry, P.L. Kendall, J.W. Thomas, Autoantigen-specific B-cell depletion overcomes failed immune tolerance in type 1 diabetes, Diabetes 61 (8) (2012), 2037-44.
[53] Zion, T.C. *IND-enabling assay validation and toxicology studies on AKS-107: a novel antigen-specific B cell therapeutic to prevent Type 1 Diabetes*. 05-05-2022]; Available from: https://grantome.com/grant/NIH/R44-DK107099-04.
[54] S.E. Hulsebosch, et al., Ultra-long-acting recombinant insulin for the treatment of diabetes mellitus in dogs, J. Vet. Intern. Med. 36 (4) (2022) 1211–1219.
[55] R.A. Maldonado, et al., Polymeric synthetic nanoparticles for the induction of antigen-specific immunological tolerance, in: Proceedings of the National Academy of Sciences of the United States of America 112, 2015, pp. E156–E165.
[56] J. Sestak, et al., Single-step grafting of aminooxy-peptides to hyaluronan: a simple approach to multifunctional therapeutics for experimental autoimmune encephalomyelitis, J. Control Release 168 (3) (2013), 334-40.
[57] J.O. Sestak, et al., Structure, size, and solubility of antigen arrays determines efficacy in experimental autoimmune encephalomyelitis, AAPS J. 16 (6) (2014), 1185-93.
[58] J.O. Sestak, et al., Codelivery of antigen and an immune cell adhesion inhibitor is necessary for efficacy of soluble antigen arrays in experimental autoimmune encephalomyelitis, Mol. Ther. Methods Clin. Dev. 1 (2014) 14008.
[59] B.L. Hartwell, et al., Antigen-specific binding of multivalent soluble antigen arrays induces receptor clustering and impedes B cell receptor mediated signaling, Biomacromolecules 17 (3) (2016) 710–722.
[60] Q. Cheng, et al., Selective depletion of plasma cells in vivo based on the specificity of their secreted antibodies, Eur. J. Immunol. 50 (2) (2020) 284–291.
[61] S. Srivastava, S.R. Riddell, Chimeric antigen receptor T cell therapy: challenges to bench-to-bedside efficacy, J. Immunol. 200 (2) (2018) 459–468.
[62] L. Zhao, Y.J. Cao, Engineered T cell therapy for cancer in the clinic, Front. Immunol. 10 (2019).
[63] E. Roselli, R. Faramand, M.L. Davila, Insight into next-generation CAR therapeutics: designing CAR T cells to improve clinical outcomes, J. Clin. Invest. 131 (2) (2021).
[64] R.C. Larson, M.V. Maus, Recent advances and discoveries in the mechanisms and functions of CAR T cells, Nat. Rev. Cancer 21 (3) (2021) 145–161.
[65] D. Mougiakakos, G. Krönke, S. Völkl, S. Kretschmann, M. Aigner, S. Kharboutli, S. Böltz, B. Manger, A. Mackensen, G. Schett, CD19-targeted CAR T cells in refractory systemic lupus erythematosus, N. Engl. J. Med. (2021).
[66] A. Mackensen, et al., Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus, Nat. Med. (2022).
[67] K.A. Hay, et al., Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy, Blood 130 (21) (2017) 2295–2306.
[68] C. Orvain, et al., Is there a place for chimeric antigen receptor-T cells in the treatment of chronic autoimmune rheumatic diseases? Arthritis Rheumatol. 73 (11) (2021) 1954–1965.
[69] C.T. Ellebrecht, et al., Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease, Sci. Transl. Med. 353 (6295) (2016) 179–184.
[70] G. Di Zenzo, et al., Immune response in pemphigus and beyond: progresses and emerging concepts, Semin. Immunopathol. 38 (1) (2016) 57–74.
[71] J. Lee, et al., Antigen-specific B cell depletion for precision therapy of mucosal pemphigus vulgaris, J. Clin. Invest. 130 (12) (2020) 6317–6324.
[72] S. Oh, et al., Precision targeting of autoantigen-specific B cells in muscle-specific tyrosine kinase myasthenia gravis with chimeric autoantibody receptor T cells, Nat. Biotechnol. (2023).
[73] K. Parvathaneni, D.W. Scott, Engineered FVIII-expressing cytotoxic T cells target and kill FVIII-specific B cells in vitro and in vivo, Blood Adv. 2 (18) (2018) 2332–2340.
[74] B. Zhang, et al., In vitro elimination of autoreactive B cells from rheumatoid arthritis patients by universal chimeric antigen receptor T cells, Ann. Rheum. Dis. 80 (2) (2021) 176–184.
[75] D.T. Domoto, P.W. Askenase, H-2-dependent cell-mediated immunity in vivo: delayed-type hypersensitivity and contact sensitization induced by fluorescein isothiocyanatate-conjugated cells, J. Immunol. 125 (5) (1980) 2161–2166.
[76] K.J. Scalapino, et al., Suppression of disease in New Zealand Black/New Zealand white lupus-prone mice by adoptive transfer of ex vivo expanded regulatory T cells, J. Immunol. 177 (3) (2006), 1451-9.
[77] J. Carnahan, et al., Epratuzumab, a CD22-targeting recombinant humanized antibody with a different mode of action from rituximab, Mol. Immunol. 44 (6) (2007), 1331-41.
[78] J.H. Esensten, et al., Regulatory T-cell therapy for autoimmune and autoinflammatory diseases: the next frontier, J. Allergy Clin. Immunol. 142 (6) (2018) 1710–1718.
[79] Q. Tang, et al., In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes, J. Exp. Med. 199 (11) (2004), 1455-65.
[80] K.V. Tarbell, et al., CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes, J. Exp. Med. 199 (11) (2004), 1467-77.
[81] L.A. Stephens, K.H. Malpass, S.M. Anderton, Curing CNS autoimmune disease with myelin-reactive Foxp3+ Treg, Eur. J. Immunol. 39 (4) (2009), 1108-17.
[82] M. Fransson, et al., CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery, J. Neuroinflammation 9 (1) (2012).
[83] D. Blat, et al., Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells, Mol. Ther. 22 (5) (2014), 1018-28.
[84] M. Tenspolde, et al., Regulatory T cells engineered with a novel insulin-specific chimeric antigen receptor as a candidate immunotherapy for type 1 diabetes, J. Autoimmun 103 (2019), 102289.
[85] E. Elinav, et al., Amelioration of colitis by genetically engineered murine regulatory T cells redirected by antigen-specific chimeric receptor, Gastroenterology 136 (5) (2009), 1721-31.
[86] A.H. Zhang, et al., Targeting antigen-specific B cells using antigen-expressing transduced regulatory T cells, J. Immunol. 201 (5) (2018) 1434–1441.
[87] A.P. Pohl, et al., Suppression of FVIII-specific memory B cells by chimeric BAR receptor-engineered natural regulatory T cells, Front. Immunol. 11 (2020) 693.
[88] X. Zhou, et al., Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo, Nat. Immunol. 10 (9) (2009), 1000-7.
[89] B. Philip, et al., A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy, Blood 124 (8) (2014), 1277-87.
[90] J. McGovern, et al., Forced Fox-P3 expression can improve the safety and antigen-specific function of engineered regulatory T cells, J. Autoimmun. 132 (2022), 102888.
[91] J.H. Cho, P.K. Gregersen, Genomics and the multifactorial nature of human autoimmune disease, N. Engl. J. Med. 365 (2011) 1612–1623.
[92] M. Volkov, et al., Comprehensive overview of autoantibody isotype and subclass distribution, J. Allergy Clin. Immunol. 150 (5) (2022) 999–1010.
[93] C. Krienke, L. Kolb, E. Diken, M. Streuber, S. Kirchhoff, T. Bukur, Ö. Akilli-Öztürk, L.M. Kranz, H. Berger, J. Petschenka, M. Diken, S. Kreiter, N. Yogev, A. Waisman, K. Karikó, Ö. Türeci, U. Sahin, A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis, Science 371 (2021) 145–153.
[94] L.E. Bartels, et al., Local and systemic reactogenicity of COVID-19 vaccine BNT162b2 in patients with systemic lupus erythematosus and rheumatoid arthritis, Rheumatol. Int. 41 (11) (2021) 1925–1931.
[95] C.D. Moorman, S.J. Sohn, H. Phee, Emerging Therapeutics for Immune tolerance: tolerogenic vaccines, T cell therapy, and IL-2 therapy, Front. Immunol. 12 (2021), 657768.
[96] J. Schwaber, E.P. Cohen, Human x mouse somatic cell hybrid clone secreting immunoglobulins of both parental types, Nature 244 (1973) 444–447.
[97] H. Staudinger, *Über Polymerisation.* in *Deutschen Chemischen Gesellschaft*, Chem. Institut der Eidgen. Techn. Hochschule, Zürich, 1920.
[98] B.L. Hartwell, et al., Antigen-specific binding of multivalent soluble antigen arrays induces receptor clustering and impedes B cell receptor mediated signaling, Biomacromolecules 17 (3) (2016), 710-22.
[99] R.P. Feynman, Plenty of Room at the Bottom, American Physical Society, Pasadena, 1959.
[100] R.A. Maldonado, et al., Polymeric synthetic nanoparticles for the induction of antigen-specific immunological tolerance, Proc. Natl. Acad. Sci. U S A 112 (2) (2015). E156-65.
[101] C.H.J. Ford, et al., Localisation and toxicity study of a vindesine-anti-CEA conjugate in patients with advanced cancer, Br. J. Cancer 47 (1983) 035–042.
[102] Administration, U.S.F.D. *Drug approval package - myelotarg injection*. 05-05-2022]; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/21174_Mylotorg.cfm.
[103] Administration, U.S.F.D. *Biologic license application: 103575*. 05-05-2022]; Available from: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=103575.
[104] G. Gross, T. Waks, Z. Eshhar, Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity, Proc. Natl. Acad. Sci. USA 86 (1989) 10024–10028.
[105] Z. Eshhar, et al., Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the γ

or ζ subunits of the immunoglobulin and T-cell receptors, Proc. Natl. Acad. Sci. USA 90 (1993) 720–724.
[106] R.J. Brentjens, et al., CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia, Cancer Immunotherapy 5 (177) (2013).
[107] K.G. MacDonald, et al., Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor, J. Clin. Invest. 126 (4) (2016), 1413-24.