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Review

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Setting the target for pemphigus vulgaris therapy

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Despite the rising incidence of autoimmunity, therapeutic options for patients with autoimmune disease still rely on decades-old immunosuppressive strategies that risk severe and potentially fatal complications. Thus, novel therapeutic approaches for autoimmune diseases are greatly needed in order to minimize treatment-related toxicity. Such strategies would ideally target only the autoreactive immune components to preserve beneficial immunity. Here, we review how several decades of basic, translational, and clinical research on the immunology of pemphigus vulgaris (PV), an autoantibody-mediated skin disease, have enabled the development of targeted immunotherapeutic strategies. We discuss research to elucidate the pathophysiology of PV and how the knowledge afforded by these studies has led to the preclinical and clinical testing of targeted approaches to neutralize autoantibodies, to induce antigen-specific tolerance, and to specifically eliminate autoreactive B cells in PV.

Pemphigus as a model autoantibody-mediated disease

Autoimmunity is an incurable condition affecting 5%–8% of the Western population (1). For decades, the standard of care has relied on chronic immunosuppression, which causes significant morbidity and mortality (2–4). The ideal therapy would eliminate only disease-causing autoimmune cells while sparing immune cells that provide protective immunity, a strategy that is commonly referred to as “targeted therapy.” Pemphigus vulgaris (PV) is among the best-characterized human autoimmune diseases with regard to immune repertoire profiling and the well-defined nature of the disease autoantigen, which makes it an ideal disease to develop targeted therapeutic approaches.

PV is a potentially fatal antibody-mediated autoimmune disease that is characterized by loss of cell adhesion (also known as acantholysis) in skin and mucous membranes (Figure 1) (5). Diagnosis is readily made by histology, which shows a pathognomonic suprabasal epithelial blister characterized by a “row of tombstones,” representing keratinocytes that have lost intercellular contact due to disruption of desmosome adhesion but remain attached to the basement membrane zone by means of their hemidesmosomes. Autoantibodies in PV target the desmosomal cadherin desmoglein 3 (DSG3) in stratified epithelia, as well as DSG1 in mucocutaneous disease (6), and their binding to the keratinocyte cell surface can be clinically documented by direct immunofluorescence studies on patient skin samples or by indirect immunofluorescence studies using patient serum on various epithelial substrates. The presence of serum DSG3 autoantibodies identifies PV patients with a specificity and sensitivity of 98%–100% (7, 8); thus, nearly all PV patients but no unaffected individuals demonstrate detectable DSG3 reactivity.

Although autoreactivity to various other autoantigens in pemphigus has been described previously (9), DSG3 autoantibodies represent the major etiologic culprit of the disease, as several lines of evidence have shown they are both necessary and sufficient for the induction of acantholysis. Passive transfer of PV IgG into mice causes suprabasal skin blistering similar to that in human disease (10), which can also be induced by transfer of affinity-purified DSG3-specific antibodies and is abrogated after depletion of these antibodies from PV sera (11, 12). Autoantibodies against other autoantigens may also synergize with anti-DSG3 antibodies to cause epidermal damage (13). Collectively, these data establish PV as a model autoantibody-mediated disease, given that the disease autoantigen in PV is well defined, the pathogenic role of anti-DSG3 antibodies in PV has been clearly established, and the diagnosis of the disease is straightforward, with commercially available histology, immunofluorescence, and ELISA studies.

Conflict of interest: A.S. Payne and C.T. Ellebrecht are inventors on patents related to targeted therapy of autoimmune diseases (PCT/US15/28872), including pemphigus. A.S. Payne has received research funding from Sanofi for the development of cellular immunotherapies for pemphigus.

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Figure 1. Diagnosis of pemphigus vulgaris. Wide-spread skin blisters and crusted erosions cause pain, itching, and risk of infection (top). Diagnosis of PV is based on histology, which shows a classic “row of tombstones,” representing loss of basal keratinocyte adhesion from suprabasal keratinocytes (bottom left), and immunofluorescence analysis of patient skin or serum samples, which documents binding of autoantibodies to the keratinocyte cell surface (bottom right). $\times 400$ magnification for bottom panels.

Pathophysiologic mechanisms in PV

As opposed to other autoantibody-mediated skin diseases, such as bullous pemphigoid or epidermolysis bullosa acquisita, monovalent autoantibody fragments are sufficient to induce acantholysis in animal and human skin models, indicating that neither Fc γ receptor engagement nor complement activation is required for blister formation (14, 15). This observation is further underscored by the ability of autoantibodies to cause blisters in complement-deficient mice (16) as well as the predominance of IgG4 among PV autoantibodies (17, 18), a subclass that does not activate complement (19) and poorly binds to Fc receptors (20). Autoantibody binding to DSG3 causes skin blisters through several mechanisms, including direct interference with desmosomal adhesion by binding residues involved in trans- and cis-adhesive interactions (21–23), prevention of desmosome assembly or promotion of disassembly through clustering and/or endocytosis of DSG3 (24–27), and secondary activation of signal transduction events in keratinocytes, which augments the blistering response (28–31).

Because the autoantibody variable regions are sufficient for pathogenicity, much effort has been devoted to cloning immune repertoires from patients and PV model mice in order to characterize the autoreactive B cell populations (15, 21, 23, 32–34). All patient-derived anti-DSG3 mAbs whose epitopes have been reported bind to the amino-terminal extracellular cadherin (EC) domains, most often EC1 and EC2, where residues important for trans- and cis-adhesion reside (35, 36). The majority of B cell clones identified to date show patterns of somatic mutation consistent with an antigen-driven process (15, 23, 33), although some B cell clones, particularly those using the antibody heavy chain gene VH1-46, demonstrate few somatic mutations and some do not require those mutations to bind to DSG3 (34). The genetic and functional characterizations of B cell repertoires in PV have provided not only a deeper mechanistic understanding of PV pathogenesis, but also valuable *in vivo* data on the diversity of human anti-DSG B cells, which have facilitated the development of targeted therapeutic approaches in PV.

Regarding the autoreactive T cell repertoire in PV, DSG3-specific Th2-type CD4⁺ T cells that recognize immunodominant DSG3 peptides have been identified in PV patients (37, 38). The strongest genetic determinant of PV susceptibility is in the HLA locus (39), most notably HLA-DRB1*04:02 in Jewish individuals (40), DQB1*05:03 in non-Jewish individuals of mixed European descent (41, 42), and DRB1*14 and DQB1*05:03 in Japanese individuals (43, 44). HLA-DRB1*04:02 has been shown to bind an immunodominant DSG3 peptide (amino acids 190–204) in a manner particularly suitable for T cell activation (45). Interestingly, DSG3-specific Th1 cells can not only be detected in PV patients, but also in healthy individuals (38), whereas IL-10–producing DSG3-specific T cells with a regulatory phenotype are decreased in PV patients compared with healthy controls (46). DSG3 is expressed in the thymus under the *AIRE* promoter (47), which normally should delete DSG3-reactive T cells during central tolerization. These data suggest that peripheral tolerance can compensate for leaky central tolerance mechanisms to protect against DSG3 autoreactivity.

Current PV treatment and implications for the development of targeted therapies

In the presteroid era, the vast majority of PV patients died from their disease (48). A more recent population-based study indicates the 1-year mortality for pemphigus is 12%, representing a 3.4 hazard ratio for death in PV patients compared with age- and sex-matched controls (4). Current treatment strategies include topical and systemic corticosteroids in combination with other immunosuppressants,

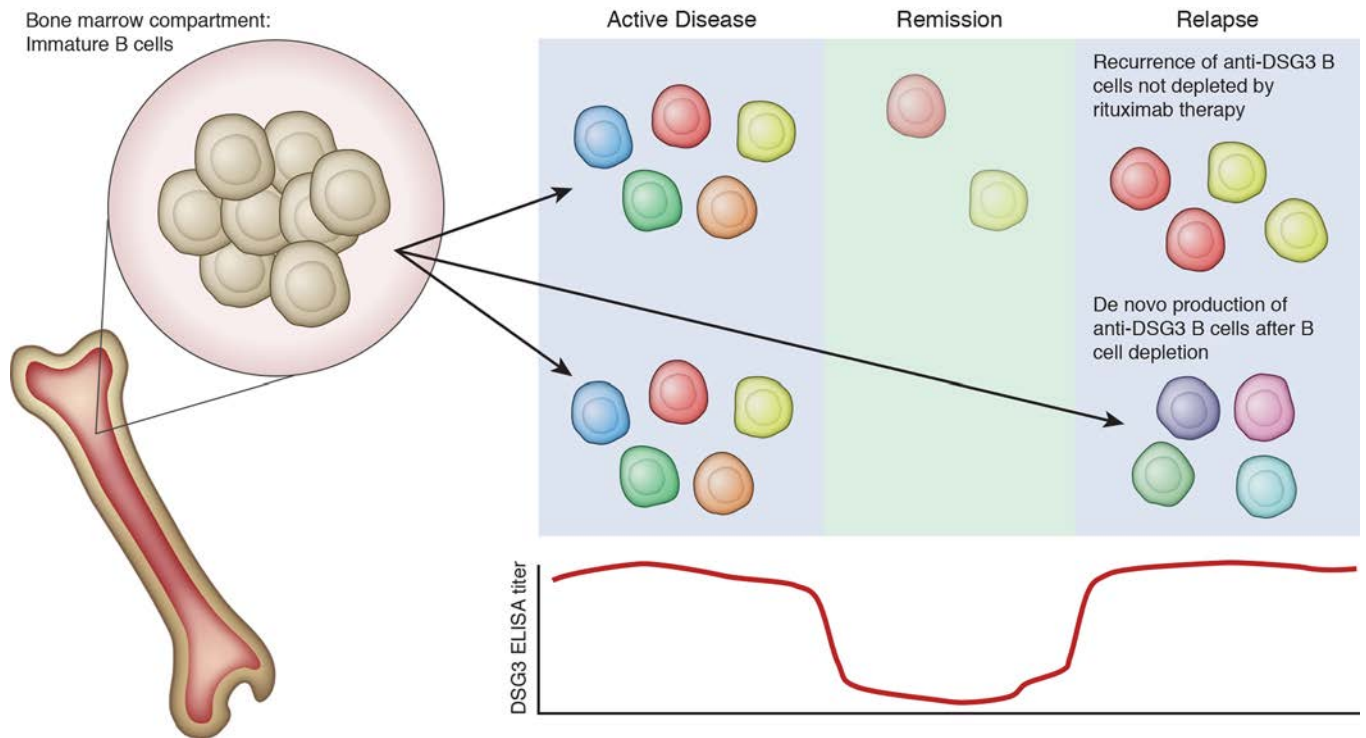


Figure 2. Potential mechanisms of disease relapse after B cell depletion therapy. Relapse after rituximab therapy of pemphigus vulgaris may be due to incomplete B cell depletion and recurrence of the same anti-DSG3 B cells observed during initial disease, a model best supported by the current data. Alternatively, disease relapse may be triggered by the appearance of a new anti-DSG3 B cell repertoire after complete B cell depletion. Production of anti-DSG3 antibodies by long-lived plasma cells, which are CD20⁻ and hence not targeted by rituximab, appears not to play a significant role for many patients, given the serologic remissions of disease observed after rituximab therapy. Illustrated by Rachel Davidowitz.

most often azathioprine or mycophenolate, although only a few randomized clinical trials have proven their efficacy (49, 50). Treatment can be augmented with intravenous immunoglobulin (51) or plasmapheresis (52–54), which aims to reduce circulating autoantibody levels. Treatment with the anti-CD20 antibody rituximab has been shown to be effective in inducing short-term disease remission in 95%–100% of PV patients (55, 56) associated with a decrease in anti-DSG antibody titers, while antibody titers to tetanus toxoid or Pneumococcal polysaccharides remain constant (57–59). This is a particularly interesting finding, as it indicates that DSG3 autoantibodies are produced by short-lived plasma cells that are continuously replenished from the CD20⁺ memory B cell pool. However, 81% of pemphigus patients experience disease relapse after rituximab (55), and analyses of rituximab use in pemphigus and other autoimmune diseases demonstrated a 1.3%–1.9% rate of fatal infection (60, 61), indicating the need for safer and more durable therapies.

Disease relapse after rituximab therapy can be explained by incomplete B cell depletion in bone marrow and secondary lymphoid organs (a known limitation of rituximab efficacy; ref. 62), by the emergence of newly formed DSG3-autoreactive B cell clones during B cell repopulation after complete B cell depletion, or by the production of autoantibodies by long-lived plasma cells that are CD20 negative and hence unaffected by CD20-targeted B cell depletion. If disease relapse is caused by the persistence and reexpansion of autoreactive B cells that are not effectively eliminated by rituximab, then strategies to completely eliminate DSG3-reactive B cells are likely to induce long-term remission in PV patients, since new anti-DSG3 B cells would typically not form. Conversely, if PV patients have an ongoing defect in tolerance to DSG3, new anti-DSG3 B cell clones would be expected to develop during each relapse after rituximab therapy. In this more challenging scenario, perpetual therapy would be required to control the development of autoreactive B cells (diagrammed in Figure 2). Additionally, if long-lived plasma cells contribute to anti-DSG antibody formation, then serologic remissions after rituximab therapy would not be observed, as this population would survive rituximab therapy, causing persistent blistering and elevations of serum autoantibody titers.

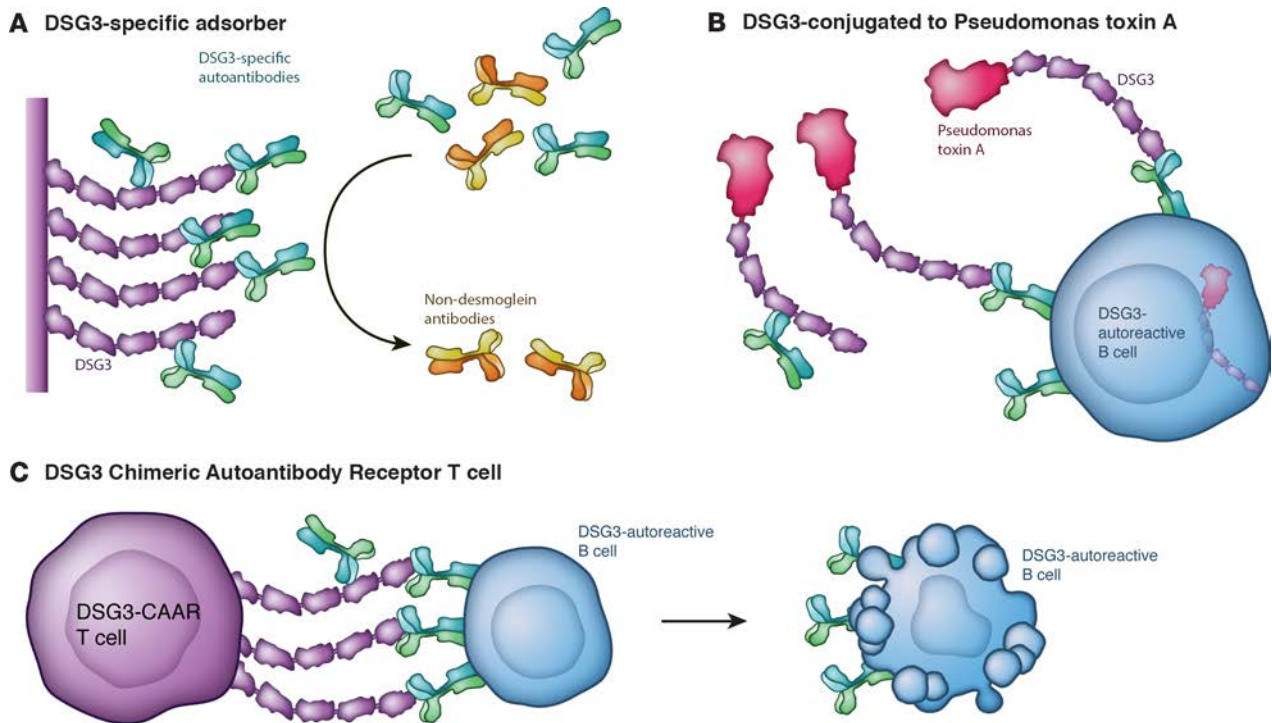


Figure 3. Autoantigen-based targeted therapies in pemphigus. Select strategies are illustrated that employ the autoantigen as a method for specific depletion of serum autoantibodies by immunoadsorption (A), targeted cell death of anti-DSG3 B cells by infusion of toxin-conjugated autoantigens that bind to anti-DSG3 B cell receptors (B), or durable elimination of anti-DSG3 B cells using DSG3-based cellular immunotherapy, also known as chimeric autoantibody receptor (CAAR) T cell therapy (C). Illustrated by Rachel Davidowitz.

Several lines of evidence suggest that the first scenario, in which there is a finite cohort of autoreactive B cells that persists over time to cause disease relapse, applies in PV. Longitudinal epitope-mapping studies of PV sera have shown that the targeted epitopes in DSG3 stay the same during active disease and relapse (63). Epitope spreading therefore appears to be a rare event, underscoring the static nature of the DSG3-reactive B cell repertoire in PV. Furthermore, cloning of DSG3-reactive B cell repertoires over time demonstrated that the same anti-DSG3 B cell clones are found during active disease and disease relapse, indicating that new autoreactive B cells generally do not appear over time and anti-DSG3 B cells are no longer detectable in the peripheral blood once patients achieve long-term remission (64). Finally, despite disease relapse in most rituximab-treated patients, PV patients can achieve both clinical and serologic remission after repeat courses of rituximab, indicating that CD20⁺ long-lived plasma cells do not appear to significantly contribute to autoantibody production, perhaps more so in patients treated early in the course of their disease (55, 59).

Taken together, the basic, translational, and clinical research studies in the field have considerably expanded our understanding of PV pathogenesis and positioned PV to be a model autoimmune disease for the development of targeted therapeutic strategies that can avoid systemic immunosuppression. Below, we discuss strategies to therapeutically target various elements of the PV autoimmune response without affecting the immune system as a whole, which would ideally prevent the severe side effects and deaths associated with current PV therapy.

Autoantibody targeting strategies

Because the antigenic specificity of the autoantibodies in PV is so well defined, strategies to either counteract or deplete these autoantibodies have been explored. Based on the knowledge that the amino-terminal epitopes of DSG3 are predominantly targeted by PV autoantibodies (11, 63), a tandem cyclic peptide consisting of DSG1 residues 81–87, highly homologous to the comparable DSG3 adhesive domain, was synthesized and shown to stabilize the trans-adhesive interface of both DSG3 and DSG1, thereby preventing autoantibody-mediated disruption of desmosomes (30, 65). This treatment was effective in preventing keratinocyte pathology *in vitro* and also epidermal blistering when applied topically *in vivo*. Additionally,

the application of the tandem peptide prevented downstream signaling events that are usually observed after autoantibody binding. In a clinical setting, this strategy would serve as an adjunctive therapy to bolster keratinocyte adhesion in response to PV autoantibodies, but disease remission would still depend on standard immunosuppressive therapy to reduce the circulating autoantibodies. A potential drawback of such a strategy would be the possible immunogenicity of the tandem peptide, which could result in antibody-mediated neutralization. Additionally, since the tandem peptide was designed to mimic the DSG trans-adhesive interface, newly formed antibodies could theoretically exacerbate acantholysis by stimulating the autoimmune response against a critical DSG3 functional domain.

As an alternative approach to autoantibody-targeted therapy, the finding that affinity-mediated adsorption of DSG3 autoantibodies from PV sera abrogates disease induction (12) led to the development of immunoabsorption columns conjugated to DSG3 or DSG1 and truncated variants spanning EC1 and EC2 to extract PV autoantibodies from patients' plasma (Figure 3A) (66). Despite concentration of targeted epitopes in EC1-2, the best depletion of plasma antibodies was observed when full-length DSG3 and DSG1 were used for immunoabsorption, suggesting that conformational epitopes recognized by PV autoantibodies (67) were not sufficiently reproduced when using their truncated DSG fragments. However, a construct consisting of only DSG1 EC1 and EC5 was recognized by 12 of 13 pemphigus foliaceus (PF) patient sera, consistent with the observation that an EC1-targeted mAb has been shown to inhibit binding of the majority of polyclonal PF sera (68).

Using antigen-specific immunoabsorbers that exclusively extract the disease-causing autoantibodies represents an attractive approach to therapy, as it avoids hypogammaglobulinemia associated with non-specific immunoabsorption strategies and hence the risks of generalized immunosuppression. An additional advantage of antigen-specific immunoabsorption is the reduction in complications due to fibrinogen fluctuation after the procedure compared with plasma exchange (69). An unanswered question in the context of antigen-specific immunoabsorption is whether release of the autoantigen from the adsorber into the serum could modify the immune response to DSG3, either through a tolerizing and therefore beneficial effect or a deleterious exacerbation of the immune response against DSG3. Both outcomes appear possible, as studies have shown that autoantigen presentation without costimulatory signals results in tolerance induction; however, tolerance-inducing strategies using peptide or otherwise modified autoantigens can induce disease flares in other autoimmune conditions (70).

Another approach that has been explored to target the soluble DSG3-specific autoantibodies in PV utilizes anti-idiotypic antibodies (71) raised in rabbits by immunization with monoclonal anti-DSG single-chain variable fragments cloned from PV patients. As shown by ELISA and keratinocyte dissociation assays, pooled anti-idiotypic antisera inhibited antibody binding to DSG3 and acantholysis induced by the serum IgG of the patient from whom the antibodies were cloned but not serum IgG from two other PV patients. Nevertheless, pathogenic serum IgG was eluted from the antisera-conjugated immunoabsorption columns from all 3 PV patients, suggesting that this approach identified some but not all of the pathogenic antibodies in other PV patients (71). These data indicate that the B cell idiotypes in PV are likely too diverse to be therapeutically targeted in all patients either through a specific anti-idiotypic or through more general VH gene-targeted reagents, although personalized approaches to identify the scope of B cell idiotypes within each patient could be effective. These reagents could be utilized in several therapeutic applications: as a method of anti-DSG antibody adsorption, as soluble mAbs that would work similarly to rituximab and provide the advantage of only depleting anti-DSG B cell populations through their surface B cell receptor (BCR), or as chimeric immunoreceptors that direct T cell cytotoxicity against specific B cell populations (discussed further below). A drawback of using such reagents as soluble infused antibodies is the formation of immune complexes between DSG3-specific and anti-idiotypic antibodies, which could exacerbate the autoimmune response or cause other immune complex-mediated complications (72).

Antigen-specific tolerance induction

As antigen-specific autoantibody production is thought to result from T cell stimulation of B cells, efforts to induce antigen-specific tolerance hold promise as methods to interfere with all aspects of the detrimental immune response. However, there are challenges facing this approach, particularly in autoantibody-mediated diseases in which immune complex formation between soluble autoantibodies and administered autoantigen could result in immune complex-mediated exacerbation of disease. Efforts to induce tolerance in various autoimmune diseases by administration of autoantigen have shown variable efficacy and some flares

of disease (70). One notable success is in multiple sclerosis (MS), which has led to a phase I clinical trial in humans using peripheral blood mononuclear cells (PBMCs) chemically engineered to present peptides derived from presumptive MS autoantigens in the absence of costimulatory signals (73). The trial demonstrated the safety and tolerability of this strategy, and the patients showed a lower frequency of antigen-specific T cells after treatment with increasing doses of tolerizing PBMCs, suggesting that pathogenic T cells had been deleted.

In PV, one human clinical trial has explored the safety and feasibility of a peptide vaccine strategy to induce DSG3-specific immune tolerance (74). A peptide encompassing amino acids 186–204 of DSG3, predicted to specifically bind to the pocket of HLA-DRB1*0402 (45), was evaluated in a phase I trial in PV patients carrying the DRB1*0402 allele to determine if it would induce tolerance in CD4⁺ T cells through the MHCII/T cell receptor interaction in the absence of costimulatory signals. The treatment was generally safe and well-tolerated. Two of seventeen patients showed disease flares during a 5-month follow-up period; however, no significant change in autoantibody titers against DSG3 was observed, and no further clinical development of this strategy has been pursued.

Although not tested in PV, it is worthwhile to discuss the clinical success of allergen-specific tolerance induction by systemic desensitization (75) and whether similar approaches could work in PV. Exposure to increasing doses of allergen has been shown to divert the B cell response from anaphylaxis-causing IgE to blocking IgG4 antibodies that lack effector function (76, 77). However, as discussed above regarding the pathophysiology of pemphigus, such a strategy may not work in PV since acantholysis induced by PV autoantibodies does not depend on the antibody Fc. Moreover, since the majority of PV autoantibodies already belong to the IgG4 subclass, a deviation of the immune response by chronic antigen stimulation to IgG4 is not likely to ameliorate and may worsen the course of PV.

An intriguing strategy for antigen-specific tolerization may be the utilization of autoantigen-specific Tregs that suppress DSG3-specific immune responses, an approach supported by the finding of decreased DSG3-specific Tregs in PV patients relative to unaffected individuals (46). Tregs that suppress HLA-specific T cell responses by means of an anti-HLA chimeric antigen receptor (CAR) have shown promise in preclinical studies (78), although strategies to avoid broader-than-intended immunosuppression must be considered.

Antigen-specific B cell depletion

Since anti-DSG B cells produce the disease-causing antibodies in PV, targeting antigen-specific B cells represents an extremely attractive approach that is likely to be effective, given the short-term efficacy of CD20-mediated B cell depletion in PV, and much safer than other B cell depletion strategies due to the preservation of non-DSG-reactive B cells by this approach. As discussed previously, short-lived plasma cells are the key antibody-secreting cells in PV and are dependent on the CD20⁺ B cell pool for replenishment. Mature CD20⁺ B cells express BCRs on their surface, consisting of the same IgG molecules that the B cell will secrete once activated to mature into a plasma cell, except membrane anchored by means of a transmembrane domain. BCRs mediate the activation of the B cell upon antigen encounter, and this activation is associated with internalization of the BCR together with the bound antigen followed by processing of the antigen and presentation of the antigen-derived peptides in the context of MHCII to CD4⁺ T helper cells (79). PV-specific B cells will express a DSG3-specific BCR on their surface, which labels them unequivocally as autoreactive in PV, and can be exploited for antigen-specific B cell targeting. As discussed previously, prior clinical and translational studies on rituximab efficacy in pemphigus predict that such an approach should effectively eliminate the critical autoreactive B cell populations in PV, resulting in both clinical and serologic disease remission.

An initial study targeting the BCRs of DSG3-specific B cells in PV was performed using chimeric proteins consisting of the DSG3 extracellular domains fused to *Pseudomonas* exotoxin A (PE) (80). PE mediates its toxic effect by ADP-ribosylation of elongation factor 2 (EF2), which inhibits ribosomal protein synthesis in the host cell (81, 82). Binding of DSG3-PE to anti-DSG3 BCRs triggers internalization and processing of PE, resulting in inhibition of protein translation in anti-DSG3 B cells and thus targeted cell death (Figure 3B). Incubation of DSG3-PE with mouse hybridomas that express surface anti-DSG3 BCRs, as well as B cells derived from DSG3-immunized mice, resulted in a 40%–60% reduction in anti-DSG3 B cells after 48 hours. Killing was enhanced by inclusion of a carboxy terminal KDEL sequence that facilitates trafficking of proteins to the endoplasmic reticulum (83). The incomplete killing of anti-DSG3 B cells by DSG3-PE was postulated to be due to incomplete processing of the toxin and antibody-mediated

neutralization of the soluble chimeric construct, the latter of which presents a major *in vivo* challenge to the therapeutic approach, as the soluble antibodies have to be overcome to induce a therapeutic effect at the B cell level and to avoid risks of immune complex formation. Additionally, the administration of a foreign protein such as PE can induce potent antibody responses (84), further limiting the efficacy of chimeric soluble proteins for targeted therapy.

We recently developed an approach to use the DSG3 autoantigen to target autoreactive B cells in PV by incorporating DSG3 into a chimeric immunoreceptor that can direct a patient's own T cells to specifically kill autoantigen-specific B cells (Figure 3C), a strategy known as chimeric autoantibody receptor (CAAR) technology (85). CAARs were developed by re-engineering CARs, which had previously shown success for cancer therapy. CARs consist of an antigen-specific antibody fragment on the surface of the T cell fused to a cytoplasmic CD3 ζ signaling domain, which directs MHC-independent T cell cytolysis against cells expressing the antigen of interest (86) and whose efficacy can be potentiated through incorporation of costimulatory signaling domains derived from CD28 or CD137 (87, 88). Such approaches using anti-CD19 CAR-T cells against B cell cancers have shown remarkable clinical efficacy, with reports of long-lasting remissions in the majority of treated patients (89–93). The success of CAR-T therapy compared with soluble antibody-based approaches are in part due to the fact that CAR-T cells are activated upon target cell encounter, resulting in 10,000-fold proliferation of CAR-T cells *in vivo* (94) for potent and specific elimination of all target cells. An additional advantage of CAR-T “living therapy” is due to the incorporation of CD28 and/or CD137 costimulatory domains in the CAR design that prevent T cell exhaustion and promote persistence of memory CAR-T cells that provide continuous surveillance against cancer recurrence (87, 88, 95). In PV, the anti-DSG3 BCR specifically marks the autoreactive B cell population; thus, the DSG3 CAAR should confer potent and persistent antigen-specific B cell killing in pemphigus, leading to clinical and serologic remission.

To evaluate the feasibility of the CAAR approach (85), truncated fragments of the DSG3 extracellular domain, consisting of EC1-3, EC1-4, and EC1-5, were expressed in primary human T cells as chimeric receptors fused to CD137/CD3 ζ signaling domains. All three DSG3 CAARs were shown to be conformational, based on binding of an anti-DSG3 mAb that only recognizes mature conformational DSG3 (96). DSG3 EC1-3 and DSG3 EC1-4 CAAR T cells were shown to specifically kill murine hybridomas and human B cell lines expressing anti-DSG3 BCRs, whereas DSG3 EC1-5 CAAR-T cells did not exhibit significant target cell cytolysis. This lack of target cell cytolysis was thought to be due to the variable surface expression and/or the suboptimal length of the EC1-5 CAAR, which is predicted to create an intermembrane distance greater than that required for optimal T cell activation (97). In a PV hybridoma mouse model (21), characterized by suprabasal epithelial blistering due to hybridoma secretion of polyclonal anti-DSG3 antibodies targeting DSG3 EC1, EC2, and the EC3/4 interface, representing the broad range of B cell epitopes and BCR affinities that could be targeted in human patients, DSG3 CAAR-T cells eliminated all anti-DSG3 BCR⁺ cells, resulting in elimination of serum anti-DSG3 antibody titers and prevention of epithelial blistering. No off-target toxicity was observed against desmosome-expressing tissues or Fc γ receptor-expressing cells, which could bind anti-DSG3 antibodies and potentially become targets for CAAR-T cell cytotoxicity. Additionally, CAAR-T cells were effective in eliminating anti-DSG3 B cells both *in vitro* and *in vivo*, even in the presence of anti-DSG3 antibodies that could block DSD3 CAAR interactions or lead to DSG3 CAAR-T clearance. The effect of soluble autoantibodies on CAAR-T function is complicated, as different anti-DSG3 mAbs can have differing effects on CAAR-T function, including direct inhibition of cytotoxicity, endocytosis of DSG3 CAAR-Ts (which can be replenished by newly synthesized CAAR-Ts), or stimulation of CAAR-T proliferation and cytokine production. Collectively, given the ability of CAAR-T cells to proliferate and synthesize new CAAR-T molecules, the data suggest that soluble anti-DSG3 antibodies likely would not prevent and in fact may enhance CAAR-T function.

The DSG3 CAAR approach offers several therapeutic advantages and fewer toxicities compared with antibody-based B cell depletion or CAR-T therapy of cancer. First, CAAR T cells can expand and persist indefinitely *in vivo* to eliminate DSG3-autoreactive B cells, presumably with superior potency and durability compared with antibody-mediated B cell depletion. Second, antigen-specific B cell depletion avoids the risks of generalized immune suppression associated with pan-B cell depletion. Moreover, commonly observed side effects of CAR treatment of cancer, such as cytokine release syndrome (98, 99), are not likely to apply in the autoimmune setting, since cytokine release syndrome is associated with high tumor cell burden and the frequency of DSG3-autoreactive B cells is several log-fold lower compared with leukemia cells (100).

Consistently, tumor lysis syndrome associated with rituximab therapy of cancer has never been reported in pemphigus patients after rituximab treatment given their normal B cell counts prior to therapy, indicating that the target cell frequency may play an important role in the development of cytokine release syndrome and tumor lysis syndrome. Additionally, target cell escape in cancer has been attributed to loss of CD19 expression or mutation of CD19 (101), which is likely irrelevant in PV since B cells that shed their BCRs can no longer receive activation signals through the BCR to mature into antibody-secreting cells, and B cells that mutate their BCRs to no longer bind DSG3 by definition become irrelevant to disease. Human clinical trials will be necessary to determine whether CAAR technology can offer safe and long-lasting disease remission.

Future directions

In summary, as a result of decades of basic, translational, and clinical research, PV has emerged as one of the best-characterized human autoimmune diseases. As potent and feasible strategies for antigen-specific targeting progress from preclinical proof of concept to clinical trials, the ideal of safe and effective remissions of autoimmunity by targeting only the disease-causing immune populations is finally coming within our reach. A broad range of approaches to target the pathogenic autoantibodies or the immune cells that stimulate their production are in preclinical and clinical development for PV, offering hope that the “row of tombstones” that describes the histology of PV will eventually have only diagnostic but not prognostic significance.

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