

Increasing the Complement of Therapeutic Options in Bullous Pemphigoid



Carolyn J. Kushner¹ and Aimee S. Payne¹

Bullous pemphigoid is a potentially life-threatening autoantibody-mediated dermatosis characterized by blister formation. Experimental mouse models of bullous pemphigoid feature complement-induced inflammation and tissue damage. Kasprick et al. now provide preclinical data that utilize ex vivo human skin assays and support testing of complement inhibition as a therapeutic strategy in human bullous pemphigoid.

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Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by autoantibodies to the hemidesmosomal proteins BP180 (also known as collagen XVII) and BP230, which are expressed by basal keratinocytes abutting the epithelial basement membrane zone. The management of this potentially life-threatening dermatosis commonly involves prolonged use of systemic corticosteroids or adjunctive immunosuppressive agents, risking serious infections and other side effects. Kasprick et al. (2017) now describe laboratory investigations that support a more targeted and potentially safer therapeutic strategy for BP—namely, blocking the classical pathway of complement activation.

The authors' studies were based on the concept that blister formation in BP depends not only on autoantibody binding to skin target proteins, causing weakened cell attachment, but also on effector functions induced after autoantibody binding, including activation of complement and attraction of neutrophils, eosinophils, and/or mast cells, leading to inflammation and tissue damage at the dermal-epidermal junction. This spectrum of molecular and cellular pathology results in the clinical presentation of tense subepidermal blisters and pruritic urticarial plaques

that are classic for BP. Experimental models of BP, in which anti-BP180 IgG is passively transferred to mice, have shown that complement activation by anti-BP180 IgG leads to mast cell degranulation at the dermal-epidermal junction, neutrophil accumulation, and cleavage of BP180 by neutrophil elastase, thus contributing to subepidermal blister formation (Lin et al., 2012; Nelson et al., 2006). The critical role of complement in this process is supported by the lack of blister formation in C4 and C5 alpha receptor-deficient mice, and by delayed blister formation in mice deficient in other pathways of complement activation (Heimbach et al., 2011; Nelson et al., 2006). However, both complement-dependent and complement-independent effects of anti-BP180 IgG have been described, with the latter including internalization and hemidesmosomal depletion of BP180, as well as induction of keratinocyte IL-6 and IL-8 production (Iwata et al., 2009; Messingham et al., 2011; Ujiie et al., 2014).

With an eye toward testing the therapeutic potential of complement inhibition in human BP, Kasprick et al. now show that TNT003, a mouse anti-C1s monoclonal antibody that interferes with the classical pathway for complement activation (Figure 1), prevented BP IgG-induced complement activation

in an ex vivo human skin cryosection assay. The authors first determined if various complement components were depleted in the peripheral blood of patients with BP. They found that plasma levels of complement were similar in patients with BP compared with controls and that they did not change with disease treatment. The authors concluded that complement activation in BP is largely restricted to the skin compartment. Next, they applied complement-inactivated BP serum to normal human skin cryosections to allow BP IgG binding, followed by incubation with normal human plasma as a source of complement in the presence or absence of TNT003. They then assessed C3c deposition on cryosections, as well as C3a, C4a, and C5a anaphylatoxin formation in the assay supernatants. Only 32 of 91 BP sera activated complement in this assay, despite the fact that 61 patients demonstrated C3 staining at the basement membrane zone in direct immunofluorescence studies previously performed on patient skin biopsies for disease diagnosis. The authors attributed this discrepancy to technical issues such as serum dilution and the presence of EDTA that may have inhibited sera with lower complement-fixing activity. Using a subset of 18 complement-activating BP sera in their ex vivo assay, the authors showed that TNT003 inhibited C3c deposition at the basement membrane zone in a dose-dependent manner, as well as C4a and C5a anaphylatoxin formation in the complement activation assay, with a TNT003 dose of ≥ 10 $\mu\text{g/ml}$ preventing C3c deposition in all 18 sera tested. TNT003 did not prevent C3a anaphylatoxin formation in the assay supernatants, which the authors postulate could have been triggered by nonspecific or nonclassical pathway complement activation, given the C3c deposition observed at the stratum corneum in some skin sections.

The work by Kasprick et al. shows that early blockade of the classical complement pathway by targeting C1s inhibits C3c deposition and C4a/C5a formation after binding of BP serum IgG to human skin cryosections. These findings, together with compelling prior mouse

¹Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence: Aimee S. Payne, Department of Dermatology, University of Pennsylvania, 1009 Biomedical Research Building, 421 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA. E-mail: Aimee.Payne@uphs.upenn.edu

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Clinical Implications

- Preclinical models allow the assessment of novel therapeutic strategies for bullous pemphigoid.
- Anticomplement monoclonal antibody TNT003 blocks pathogenic mechanisms underlying bullous pemphigoid in laboratory studies.
- Preclinical and prior clinical data support testing anticomplement therapies in patients with bullous pemphigoid.

data indicating a critical role for complement in the pathogenesis of experimental BP, provide a rationale for testing TNT009 (now BIVV009), the humanized monoclonal IgG4 version of TNT003, in clinical trials involving patients with BP.

In regard to potential toxicities from C1s blockade in humans, experience to

date is limited but encouraging. TNT009 was reported to be safe and well tolerated in a first-in-human, double-blind, randomized phase 1 trial in 64 healthy volunteers, 48 of whom received TNT009 and 16 of whom received placebo (Mühlbacher et al., 2017). No serious adverse events were recorded, and no significant differences were

observed in the incidence of mild or moderate adverse events, including infections, between placebo-treated and TNT009-treated subjects despite complete classical pathway complement inhibition for more than 4 weeks in the latter group. Because of its selectivity for the classical pathway of complement, which is activated by antibody-antigen complexes, TNT009 offers preferential targeting of complement components that are thought to be more relevant to disease in BP, while sparing the alternative and lectin pathways, which can mediate innate immune defenses. This risk-benefit profile is hypothesized to be more favorable than that observed with inhibitors of terminal components of the complement cascade such as eculizumab (Figure 1), an anti-C5 monoclonal antibody, which is associated with a thousand-fold increase in the risk of meningococcal disease despite meningococcal vaccination before therapy (McNamara et al., 2017).

In addition to the potential for infectious complications from complement blockade, the risk of autoimmunity is theoretically possible with C1 inhibition, although no new onset autoimmune diseases have been reported with either TNT009 or eculizumab therapy to date. Genetic deficiencies of early classical pathway components, including C1q and C4, are associated with systemic lupus erythematosus (Macedo and Isaac, 2016). One patient receiving the highest dose of TNT009 (100 mg/kg) developed a low-level (1:80) antinuclear antibody titer at the end-of-study visit (Mühlbacher et al., 2017). Although additional studies involving more patients will be necessary to elucidate the risks of C1s blockade, on balance, the available data suggest that selective targeting of the classical complement pathway offers a promising approach to BP therapy. The FDA designated BIVV009 for orphan drug status for BP in August 2017, thus facilitating future clinical development. Human trials of BIVV009 will not only offer the potential to expand the therapeutic armamentarium in BP, but will also afford the exciting scientific opportunity to define the necessity of complement for disease pathogenesis in patients with BP.

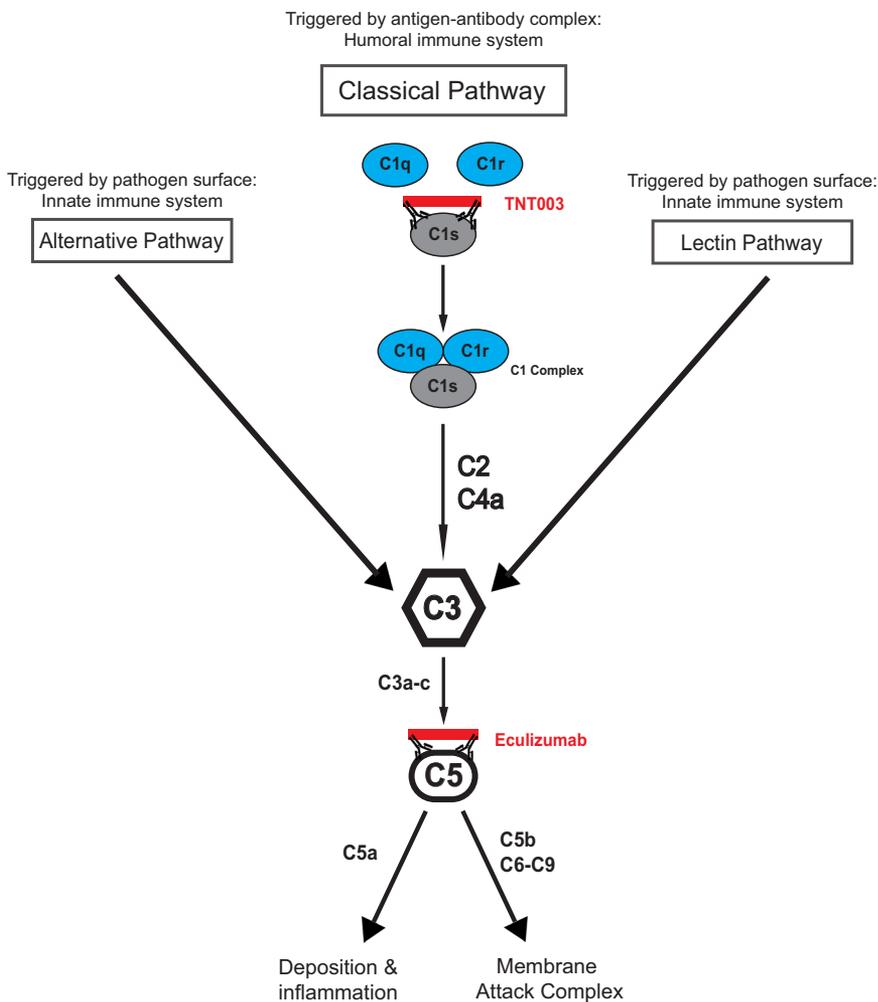


Figure 1. Mechanism of action of TNT003. TNT003 blocks classical pathway complement activation by binding C1s, leading to reduced production of inflammatory mediators (C3a, C4a, and C5a) as well as decreased C3c deposition, while sparing alternative and lectin complement activation pathways that can mediate innate immune defenses. Terminal complement blockade with the anti-C5 monoclonal antibody eculizumab inhibits the classical, alternative, and lectin pathways.

ORCID

Aimee S. Payne: <http://orcid.org/0000-0001-9389-7918>

CONFLICT OF INTEREST

ASP, equity holder in Tycho Therapeutics, focused on targeted therapy of autoimmunity; and is inventor on patent licensed to Novartis for cellular immunotherapy of antibody-mediated disease. CJK states no conflict of interest.

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mechanism known as the circadian clock that is self-sustained and oscillates with a periodic cycle of approximately 24 hours. In mammals, the molecular clock is entrained daily by the morning sunlight entering the eyes to reset the master clock located in the suprachiasmatic nucleus of the brain. The suprachiasmatic nucleus, via various hormonal and neuronal signals, synchronizes peripheral tissues including the heart, lung, liver, and skin. At the molecular level, the circadian clock is made up of primary as well as secondary feedback loops that are primarily responsible for approximately 24-hour rhythmicity of clock-controlled genes. Proteins including brain and muscle ARNT-like protein 1 (BMAL1), circadian locomotor output kaput (CLOCK), cryptochromes (CRY 1, 2), and periods (PER 1, 2, 3) make up a transcriptional-translational feedback loop that is initiated by dimerization of BMAL1 and CLOCK and the subsequent binding of this complex to the E-Box sequence (CACGTG or CACGTT) found in the promoter region of target clock-controlled genes (Lowrey and Takahashi, 2011). Tissue-specific circadian expression patterns have been observed in as many as 10% of mammalian genes, and many of these genes regulate cell cycle, metabolism, cell death, and DNA repair processes (Sancar et al., 2015).

Apart from its life-giving quality, the Sun emits harmful UVR that induces DNA damage in skin. Exposure to UVR causes a plethora of skin-related issues including photoaging, sunburn erythema, and skin cancers including melanomas, the deadliest skin cancers. In recent years, the skin's circadian clock has been shown to regulate various cellular responses after UVR exposure, including nucleotide excision repair, cell cycle checkpoints, oxidative stress, and apoptosis (Dakup and Gaddameedhi, 2017; Gaddameedhi et al., 2011; Geyfman et al., 2012; Plikus et al., 2015; Sancar et al., 2015; Wang et al., 2017). These robust protective mechanisms attenuate unwanted consequences of UVR-mediated DNA damage. Skin clock regulation of nucleotide excision repair has been well characterized using the SKH-1 hairless mouse model, where it was observed that an elevated rate of

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UV-B-Induced Erythema in Human Skin: The Circadian Clock Is Ticking



Soumyadeep Sarkar¹ and Shobhan Gaddameedhi^{1,2}

Acute exposure of skin to UV-B causes DNA damage and sunburn erythema in both mice and humans. Previous studies documented time-of-day-related differences in sunburn responses after UV-B exposure in mice. Because humans are diurnal and mice are nocturnal, the circadian rhythm in human skin was hypothesized to be in opposite phase to the rhythm in mice. A study by Nikkola et al. demonstrates that humans are more prone to sunburn erythema after evening exposure to solar UV-B radiation as compared with morning exposure.

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The majority of living organisms on the Earth are photosensitive and are influenced by the solar day and night cycle

created by the axial rotation of the Earth. Consequently, cells have developed an amazing time-keeping

¹Department of Pharmaceutical Sciences, College of Pharmacy, Washington State University, Spokane, Washington, USA; and ²Sleep and Performance Research Center, Washington State University, Spokane, Washington, USA

Correspondence: Shobhan Gaddameedhi. WSU College of pharmacy, P.O. Box 1495, Spokane, WA 99202. E-mail: shobhan.gaddameedhi@wsu.edu

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