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Hitting the Bullseye in Autoimmunity: Targeting Biologics through Tethering: Examining a Therapeutic Potential for Vitiligo and Beyond

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Biologic therapies targeting aberrant immune responses in cutaneous and systemic autoimmune diseases have had a significant impact on reducing mortality and morbidity for conditions that could not be treated with conventional drugs. However, their systemic effects increase vulnerability to infections, malignancies, and development of secondary autoimmune complications, underscoring the need for localized, targeted delivery of biologics. The article by Ying-Chao et al. (2022) titled "A keratinocyte-tethered biologic enables location-precise treatment in mouse vitiligo" highlights the potential for using bispecific antibodies (BsAbs) as a novel method of localized drug delivery through tethering, where one of the two antibody specificities is used to target a structural surface protein to anchor the biologic within the tissue. Thus, in this study, an anti–IFN-γ–neutralizing antibody was fused to a single-chain variable fragment (scFv) of a human anti-desmoglein (DSG) antibody (Px44) that was previously shown to specifically bind to the epidermal cell surface of basal and hair follicle keratinocytes (KCs) (Kouno et al., 2013). The authors found that BsAb localized specifically to the skin, was eliminated from the blood faster, and had fewer systemic effects than a nontethered version of their biologic. In a mouse model of vitiligo, injection of BsAb to a footpad successfully blocked T-cell–mediated inflammation in the skin near the injection site, showing treatment effectiveness with diminished off-target effects. Therefore, tethering of biologics is worth considering as a strategy for localized drug delivery in chronic inflammatory skin conditions and may have implications for the treatment of other autoimmune diseases.

Anchoring as a novel method to target a drug to a specific location

Whether nanoparticles or liposomes, localized drug delivery has been investigated with mixed success to reduce global off-target effects. For biologic drugs, multispecific biologics could prove to be a more successful approach. In silico modeling of bi/multispecific biologics and surface-bound receptors has been used to evaluate the potential efficacy and design aspects of this immunotherapeutic strategy (Su et al., 2020). Various scenarios were tested, including covalently tethering two ligands of immune receptors such as major histocompatibility complex and PD-L1 to target only nonnaïve T cells. This principle can be applied to a variety of formats. Thus, in addition to targeting activated T cells, one can envision tethering cytokine inhibitors, activating or inactivating enzymes, GFs, or GF inhibitors. In this study by Ying-Chao et al. (2022), a bispecific biologic approach was used to tether IFN-γ–neutralizing antibody for skin-directed anchoring through anti-DSG specificity.

DSG is a desmosomal cadherin-like adhesion molecule that maintains tissue integrity by bridging intermediate filaments in KCs. The scFvs specific for DSG were isolated from a patient with pemphigus vulgaris (Payne et al., 2005) and later repurposed (as Px44) for skin-targeted drug delivery (Kouno et al., 2013). Px44 scFv has several advantages: its small size allows for more flexibility with fusion partners, and its human origin and a lack of the effector region of a full-length antibody prevents antibody-induced inflammatory response. Furthermore, its design can be manipulated to fuse essentially any proteins with it for delivery to the epidermis. Some conceivable disadvantages include a potential immune response against the linker or foldon, which is a small trimerization domain that can be cloned in for increased biological stability, and its short half-life, albeit this potential immunogenicity and short half-life did not hamper the BsAb-mediated immune suppression in the vitiligo mice. Targeting anchors other

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Clinical Implications

- Tethering can provide a novel way for spatiotemporal control of drug delivery.
- Use of in vivo vitiligo models is a proof of concept for the therapeutic efficacy of tethering.
- Tethering of biologics has a potential for treatment of other autoimmune diseases.

than DSG may produce a different half-life and can be considered if a more sustained contact is required, or if a different location is targeted.

Potential clinical implications for vitiligo

Vitiligo, a progressive appearance of sharply demarcated white depigmented patches on the skin, is caused by T-cell–mediated killing of melanocytes in the basal epidermis. Autoreactive cytotoxic CD8+ T cells recognize melanocyte-specific antigen and secrete IFN-γ (Harris et al., 2012) that in turn induces secretion of CXCL9 and CXCL10 from the surrounding KCs (Rashighi et al., 2014), further attracting more cytotoxic T cells to the area, leading to progression and expansion of the depigmentation. IFN-γ would therefore appear to be an exciting therapeutic target in vitiligo (Figure 1). Systemic administration of neutralizing antibodies against IFN-γ is associated with significant side effects (Buchmeier and Schreiber, 1985; Dighe et al., 1994), and currently such treatment is only approved for hemophagocytic lymphohistiocytosis, a life-threatening syndrome of excessive immune activation. However, locally tethered biologics such as an IFN-γ antagonist may overcome those limitations and present a potential alternative. Ying-Chao et al. (2022) showed that epidermally tethered anti–IFN-γ BsAb retained the ability to bind to both IFN-γ and KCs in vivo, inhibited local production of CXCL9 and CXCL10 by KCs, increased the retention of anti–IFN-γ at the site of injections for up to 48 hours after treatment, decreased the recruitment of cytotoxic T cells into the treated area, and locally reduced skin depigmentation. These findings warrant further investigation into the potential suitability of this treatment strategy for clinical use.

Potential implications for other autoimmune diseases

In addition to vitiligo, the use of bispecific biologics may have implications for other autoimmune diseases. Anchoring to KCs with the anti–IFN-γ/DSG BsAb could potentially be helpful for T-cell–mediated inflammatory conditions of the skin that target a specific structure or compartment in the skin, such as hair follicles in alopecia areata and the basal layer in lichen planus. The DSG anchor could also be fused with other biologically active proteins to localize them to the epidermis. Theoretically, the tethering strategy could likewise use other anchors specific to other organs or tissues to modulate immune signaling in diseases with well-defined localization, such as graft versus host disease. Although it remains to be determined, the applicability of tethering for treating systemic autoimmune diseases that are characterized by the generation of specific autoantibodies, as is the case with systemic lupus erythematosus and Sjogren's syndrome, may be limited, although this is likely going to depend on the particulars of each disease mechanism.

Advances in sequencing, microscopy, bioinformatics, and other technologies allow faster and more precise characterization of disease mechanisms to identify novel treatment targets, and the use of biologics for treatment is expanding. Being able to localize a biologically active protein, be it a neutralizing antibody, a proapoptotic agent, or an enzyme, to a target tissue is likely to be a highly useful venue for treating these diseases in the future and at the same time limiting potential high-risk side effects related to systemic administration.

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CONFLICTS OF INTEREST

The authors state no conflicts of interest.

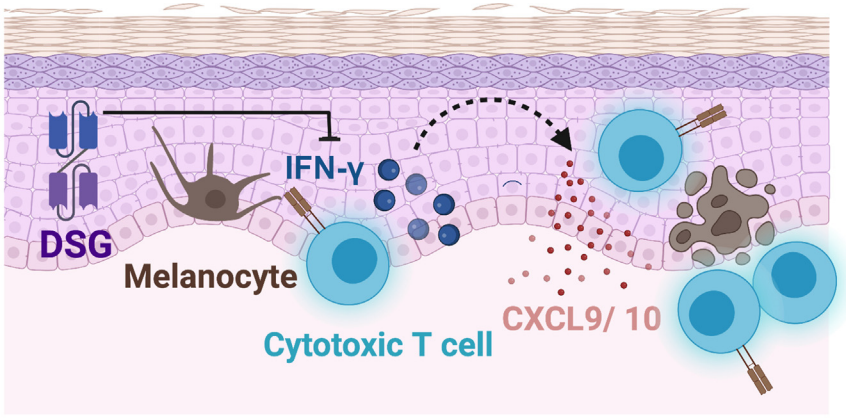

Figure 1. Epidermal tethering of anti–IFN-γ as a promising treatment option for vitiligo. The disease mechanism of vitiligo involves cytotoxic CD8 T cells secreting IFN-γ and thus stimulating keratinocytes to produce CXCL9/10 chemokines to attract more cytotoxic T cells that, in turn, kill pigment-producing melanocytes. Anchoring anti–IFN-γ BsAb that binds to DSG in basal epidermis provides a localized neutralization of the IFN-γ signaling and rescues vitiligo in mice. The figure was created with BioRender. BsAb, bispecific antibody; DSG, desmoglein.

REFERENCES

Buchmeier NA, Schreiber RD. Requirement of endogenous interferon-gamma production for resolution of Listeria monocytogenes infection. Proc Natl Acad Sci USA 1985;82:7404–8.

Dighe AS, Richards E, Old LJ, Schreiber RD. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFN gamma receptors. Immunity 1994;1:447–56.

Harris JE, Harris TH, Weninger W, Wherry EJ, Hunter CA, Turka LA. A mouse model of vitiligo with focused epidermal depigmentation requires IFN-γ for autoreactive CD8+ T-cell accumulation in the skin. J Invest Dermatol 2012;132:1869–76.

Kouno M, Lin C, Schechter NM, Siegel D, Yang X, Seykora JT, et al. Targeted delivery of tumor necrosis factor-related apoptosis-inducing ligand to keratinocytes with a pemphigus mAb. J Invest Dermatol 2013;133:2212–20.

Payne AS, Ishii K, Kacir S, Lin C, Li H, Hanakawa Y, et al. Genetic and functional characterization of human pemphigus vulgaris monoclonal autoantibodies isolated by phage display. J Clin Invest 2005;115:888–99.

Rashighi M, Agarwal P, Richmond JM, Harris TH, Dresser K, Su MW, et al. CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. Sci Transl Med 2014;6:223ra23.

Su Z, Wang B, Almo SC, Wu Y. Understanding the targeting mechanisms of multi-specific biologics in immunotherapy with multiscale modeling. iScience 2020;23:101835.

Hsueh Y-C, Wang Y, Riding RL, Catalano DE, Lu Y-J, Richmond JM, et al. A keratinocyte-tethered biologic enables location-precise treatment in mouse vitiligo. J Invest Dermatol 2022;142:3294–303.