We further transferred OT II CD4+ T cells into IvL-mOVA/Rag2KO mice, which lack both T cells and regulatory T cells (Tregs). Flow cytometric analysis revealed that recipient's regulatory T cells (Tregs) prevented autoreactive OT II CD4+ T cells proliferation. We used Ivl-mOVA transgenic mice, in which membrane-bound ovalbumin (mOVA) was expressed under the control of involucrin (Ivl) promoter. In this mouse, autoantigen (mOVA) was expressed specifically in the spinous and granular layer of the epidermis. OVA-specific CD4+ or CD8+ T cells, T cells from TCR-transgenic mice (OT II and OT I), were adoptively transferred to mice treated with or without anti-CD4 treatment. Flow cytometry analysis and two-photon microscopy revealed that OT II CD4+ T cells proliferated in the skin draining lymph nodes and infiltrated into the skin. Taken together, our study demonstrates that the CD4+ T cells were handled in the periphery in different ways and that Tregs play an essential role in the induction ofergy to autoreactive CD4+ T cells.

Real-time in vivo imaging of CD8+ T cell-mediated keratinocyte apoptosis in a graft versus host disease-like murine model

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Graft-versus-host disease (GVHD) is an epidermolytic skin disorder mediated primarily by CD8+ cytotoxic T lymphocytes (CTLs) on epidermal antigens. However, the in vivo dynamics of keratinocyte (KC) apoptosis and KC-CTL interaction in GVHD is totally unknown. To address this, we performed intravitral imaging of CD8+ T cell-mediated KC apoptosis using a novel GVHD-like model, in which membrane-bound chicken ovalbumin is expressed in KC under the control of involucrin promoter (Ivl-mOVA). Ivl-mOVA mice spontaneously exhibit cutaneous manifestations of GVHD-like disease both clinically and histologically after transfer of CD8+ T cells from OVA-specific CD8+ T cell receptor transgenic OT-I mice. We crossed Ivl-mOVA mice with OT-I transgenic mice expressing a fluorescent probe that detects caspase-3 activation (SCAT 3.1), an indicator of apoptosis, and subjected them to intravitral two-photon microscopic analysis after the transfer of OT-I cells expressing TdTomato. We found that apoptotic KCs began to appear mainly around hair follicles, where CD8+ T cells localized. Time-lapse imaging revealed radial expansion of apoptotic KCs accompanied by an increase in number of CD8+ T cell infiltration. This was followed by eventual loss of activated caspase-3 signal at the completion of apoptosis and concomitant reduction in number of T cells. Image analysis showed that activated caspase-3 signals in KCs were characterized by: 1) the spread to adjacent cell and 2) preceding direct contact with CD8+ T cells. Our study represents a novel method for visualisation of apoptosis in antigen-specific KC apoptosis and suggests the need for both KC-KC and T cell-KC interaction for spread of apoptosis signal.

Preclinical Mechanism of Action of PRN1008, a Reversible Covalent Bruton's Tyrosine Kinase Inhibitor in Clinical Development for Pemphigus

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BTK is expressed in B cells, innate immune cells such as macrophages, neutrophils and mast cells. BTK-null (BTK-/-) or BTK-deficient (BTKfl/fl) mice are viable, indicating that B-cell receptor and Fc-receptors is a promising therapeutic target for autoimmune. Unlike currently marketed BTGK, PRN1008 is a reversible covalent inhibitor designed and optimized to preferentially bind BTK versus other kinases sharing a homologous cytokine. This tailored covalent bond enables durable target occupancy with lower systemic exposures thereby reducing the potential for off-target toxicities. PRN1008 has shown rapid and durable anti-inflammatory effects in multiple animal models, via inhibition of B cell activation and blockade of antibody-mediated immune mediated inflammatory disease activation via Fc receptor signaling blockade. In skin, PRN1008 significantly improved immune complex mediated inflammation and injury in an IgG antibody (FcyR) driven acute arthus model in rats. PRN1008 inhibited mast cell degranulation and reduced neutrophil and inflammatory cytokines, supporting the role for BTK in IgG antibody (FcxR) mediated immune responses. In addition to neutralizing pathogenic antibody binding, B cell studies demonstrated that BTK inhibition also blocked antibody-dependent cell-mediated inflammatory disease activation and reduced antibody-dependent natural inflammatory. PRN1008 safely and rapidly controlled disease without corticosteroids. Overall, PRN1008 demonstrated three simultaneous MOA benefits that, combined, allow for a fast-acting and sustained response: rapid anti-inflammatory effects, neutralization of pathogenic autoanti-bodies, receptor blocking. In a 24-week preclinical human disease model, PRN1008 reduced glycolytic activity in activated T cells and this correlated with reduced IL-9, but not IL-13 expression. Conversely, enhancing glycolytic activity by increasing glucose availability increased IL-11 expression. Functionally, these manipulations of glycolytic activity were closely linked to changes in cellular energy states, as indicated by altered ATP and ADP/ATP levels, respectively. Mechanistically, reduced ATP levels after PRN1008 inhibition led to reduced phosphorylation of STAT1, a key effector factor of IL-9 T cell function.

PPARγ regulates IL-9 expression in human T cells by promoting glycolysis

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The transcription factor PPARγ has emerged as a novel regulator of pathogenic Th9 cells. We previously identified Th9 cells as a subset of PRN1008-IL-9-producing T cells that participate in acute allergic skin inflammation. In these IL-9+ Th9 cells, the transcription factor PPARγ acts as a positive modulator of IL-9 expression through mechanisms that remain unknown. Since PPARγ is a known regulator of cellular metabolism, we hypothesized that PPARγ controls cytokine expression via modulation of T cell metabolism. Indeed, inhibition of PPARγ reduced glycolytic activity in activated T cells and this correlated with reduced IL-9, but not IL-13 expression. Conversely, enhancing glycolytic activity by increasing glucose availability increased IL-11 expression. Functionally, these manipulations of glycolytic activity were closely linked to changes in cellular energy states, as indicated by altered ATP and ADP/ATP levels, respectively. Mechanistically, reduced ATP levels after PPARγ inhibition led to reduced phosphorylation of STAT1, a key effector factor of IL-9 T cell function.