Peripheral tolerance against autoreactive CD4+ T cells and CD8+ T cells is inactivated differentially by Foxp3+ regulatory T cells

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OBJECTIVE

In order to suppress self-reactive T cells that escape from central tolerance, additional mechanisms in the immature periphery, so-called peripheral tolerance, is essential; however, this mechanism is not fully elucidated. To clarify this, here we compared the mechanisms of peripheral tolerance induction against autoreactive CD4+ T cells and against autoreactive CD8+ T cells. We used NOD.mOVA transgenic mice, in which membrane-bound ovalbumin (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 I and OT II), respectively, were intravenously injected into NOD.mOVA mice, in which mOVA was expressed specifically in the spinous and granular layer of the epidermis. OT II CD4+ T cells proliferated in the skin draining lymph nodes, while transferred OT II CD4+ T cells did not proliferate at all. We hypothesized that recipient’s regulatory T cells (Tregs) prevented autoreactive OT II CD4+ T cells proliferation. We further transferred OT II CD4+ T cells into NOD.mOVA/Rag2KO mice, which lack both T cells and B cells, or NOD.mOVA/Foxp3-diphtheria toxin receptor (DTR) mice, in which Foxp3+ Tregs can be depleted by the administration of diphtheria toxin. We found that OT II CD4+ T cells could not be activated in the absence of Tregs.

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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OBJECTIVE

Graft-versus-host disease (GVHD) is an epidermal 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 interactions in GVHD is totally unknown. To address this, we performed intravital 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 T cell receptor transgenic OT-I mice. We cross-hybridized Ivl-mOVA mice with transgenic mice expressing a fluorescent indicator that detects caspase activation (SCAT 3.1), an indicator of apoptosis, and subjected them to intravital 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 colocalized. Time-lapse imaging revealed rapid 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 model for visualization of apoptosis in antigen-specific KC apoptosis and suggest 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 is also upregulated in B cell acute lymphocytic leukemia and B cell leukemia. BTK is an essential member of the B cell receptor and Fc-receptors and is a promising therapeutic target for autoimmune. Unlike currently marketed BTKi, PRN1008 is a reversible covalent inhibitor designed and optimized to preferentially bind BTK versus other kinases sharing a homologous cysteine. 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 blockage of antibody mediated immune responses. Cell-based activation via Fc receptor signaling blockade. In skin, PRN1008 significantly improved immune complex mediated inflammation and injury in an IgG antibody (IgG) driven arthus model in rats. PRN1008 inhibited mast cell degranulation in vitro. In a cutaneous anaphylaxis model, supporting the potential for BTK in IgE antibody (FcεR) mediated immune responses. In addition to neutralizing pathogenic antibody signaling, B cell studies demonstrated that BTK inhibition also blocked antibody-dependent cell-mediated cytolysis of antibody-dependent natural killer (ADNKC) cell mediated cytolysis. PRN1008 safely and rapidly controlled disease without corticosteroids. Overall, PRN1008 demonstrates three simultaneous MOA benefits that, combined, allow for a fast-acting and sustained response: rapid anti-inflammatory effects, neutralization of pathogenic autoantibody production, respectively. In vivo, PRN1008 is also effective in mouse models of pemphigus vulgaris (PV), a severe, chronic autoimmune blistering skin disease. In these models, PRN1008 treatment allowed for a significant reduction in clinical signs of disease, as measured by a decrease in ear thickness, perilesional blistering, and stratum corneum thickness. In addition, PRN1008 was effective in a mouse model of pemphigus foliaceus (PF), a common form of pemphigus that affects the skin. In this model, PRN1008 treatment significantly reduced clinical signs of disease, such as ear thickness, perilesional blistering, and stratum corneum thickness. Overall, these data suggest that PRN1008 has potential as a treatment for pemphigus, a severe, chronic autoimmune blistering skin disease.

PPARγ regulates IL-9 expression in human T,2 cells by promoting glycolysis

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OBJECTIVE

The transcription factor PPARγ has emerged as novel regulator of pathogenic T,2 cells. We used the recently identified "T,9" cells as a subset of PPARγ+ IL-9-producing T,2 cells that participate in autoimmune skin inflammation. In these IL-9+ T,2 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,2 cells and this correlated with reduced IL-9, but not IL-13 expression. Conversely, enhancing glycolytic activity by increasing glucose uptake and increased IL-9 expression. Since metabolic state is functionally and pharmacologically distinct, these findings suggest that PPARγ regulates IL-9 expression by modulating the metabolic state of T,2 cells.

Profiling immune cells using tissue cytometry and immune cell clusters

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OBJECTIVE

Intraepithelial immune cell composition is altered in a T cell- dependent and -independent manner in a mouse model of pemphigus foliaceus (PF). We previously developed a powerful method that utilized tissue cytometry to highlight immune cells and composed of different combinations of biomarkers of CD4 activity. In summary, we developed a method for visualization of antigen-specific KC apoptosis and suggest the need for both KC-KC and T cell-KC interaction for spread of apoptosis signal.

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