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

We further transferred OT II CD4+ T cells into IvL-mOVA/Rag2KO mice, which lack both T cell and CD8+ T cell infiltration. This was followed by eventual loss of activated caspase-3 expression, 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 IvL-mOVA/Rag2KO mice, which lack both T cells and B cells, or IvL-mOVA/Foxp3-dipherteria toxin receptor (DTR) mice, in which Foxp3+ Tregs can be depleted by the administration of dipherteria toxin. We found that OT II CD4+ T cells were deleted in the absence of Foxp3+ Tregs. Flow cytometric 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 autoreactive CD4+ and CD8+ T cells were handled in the periphery in different ways and that Tregs play an essential role in the induction of anergy 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 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 crossed Ivl-mOVA mice with transgenic mice expressing a fluorescent reporter that detects caspase-3 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 localized. 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 visualisation 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, but not in keratinocytes. BTK is an essential enzyme in B-cell receptor and Fc-receptors and is a promising therapeutic target for autoimmune disease. 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 blockade of antibody mediated immune mediated inflammatory activation via Fc receptor signaling blockade. In skin, PRN1008 significantly improved immune complex mediated inflammation and injury in an IgG antibody (iCyR) driven acute arthus model in rats. PRN1008 inhibited mast cell degranulation, reduced neutrophil and eosinophil infiltration, and reduced cytokine expression (IL-6, IL-8, GM-CSF, IL-13) in tissue and plasma. Additionally, PRN1008 inhibited murine B cell activation and cytokine production in vitro. PRN1008 has shown potential to treat B cell and autoantibody driven autoimmune disorders and is under investigation clinically.

PPARγ regulates IL-9 expression in human T2 cells by promoting glycolysis

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The transcription factor PPARγ has emerged as novel regulator of pathogenic T2 cells. We recently identified IL-9 as a T2 cell cytokine that is increased in the dermis, but not in the epidermis of an IL-9 antibody treated pemphigus mouse model. We also previously identified T2 cells as a subset of PPARδ− IL-9 producing T2 cells. We now report that PPARγ promotes IL-9 expression in T2 cells that participate in allergic skin inflammation. In these IL-9+ T2 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 and active IL-9+ T2 cells and this correlated with reduced IL-9, but not IL-13 expression. Conversely, enhancing glycolytic activity by increasing glucose uptake or lactate production increased IL-9 expression. In addition, and in a manner functionally relevant, these manipulations of glycolytic activity were closely linked to changes in cellular energy states, as indicated by altered ATP and ATP/ADP levels, respectively. Mechanistically, reduced ATP levels after PPARγ inhibition led to reduced phosphorylation of STATs, a key event in T2 cell function. IL-13+ T2 cells were more efficiently activated when cultured in the presence of IL-9 and IL-2. These data suggest that the regulatory effect of PPARγ on glucose metabolism in activated IL-9+ T2 cells and that IL-9 expression in T2 cells is specifically and closely linked to cellular energy homeostasis. Thus, PPARγ+ T2 cells might function to translate nutrient availability at the site of inflammation into an immunological signal via its expression of IL-9.