Peripheral tolerance against autoreactive CD4+ T cells and CD8+ T cells is inactivated differentially by Fospx3 regulatory T cells.

We further transferred OT II CD4+ T cells into IvL-mOVA/Rag2KO mice, which lack both T cells. Proliferation in these transgenic mice in the absence of Tregs. Flow cytometric analysis play an essential role in the induction of anergy to autoreactive CD4+ T cells. (mOVA) was expressed under the control of involucrin (Ivl) promoter. In this mouse, auto-reactive OT II T cells and 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/Fospx3-diphtheria toxin receptor (DTR) mice, in which Fospx3 Tregs can be depleted by the administration of diphtheria toxin. We found that OT II CD4+ T cells were prevented from proliferation in the Fospx3 mice. 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 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 exhibited 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 OT-I mice expressing a fluorescent probe that detects caspase-3 activity (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 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 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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The purpose of this study was to develop a method to highlight immune cells and cell clusters in histology tissue stained with single or multiple biomarkers. B6 mice were implanted (rear flank) subcutaneously with UMAMM/1.7 melanoma cells and treated with or without immunotherapy (PD-1, CTLA-4). Skin, lymph nodes, and spleens were formalin-fixed paraffin-embbeded and stained with immunohistochecmistry (IHC) or immunofluorescence (IF). Biomarkers for B cells stained by immunohistochemistry (IHC) or immunofluorescence (IF) staining were: B cell receptor (B220), CD3, CD4, CD8, FOXP3, T-bet, IL-6, Ki-67, CD123, T-bet, Foxp3, ROR(gamma), CD123, T-bet, Foxp3, ROR(gamma), CD123, T-bet, Foxp3, ROR(gamma). The results for the tissue-stained images were used to acquire image tiles for analysis. Image processing and analysis was performed using StrataQuest software (TissueGnostics). An image stitching algorithm was used on the tiled images to reconstruct the whole image. Algorithms were created to align the stained images, generate a composite image consisting of all biomarkers, isolate each cell in the composite image, and identify the positive cells in the composite image. Tissue-cytometry coupled with backgapping into the tissue images was used to visualize, quantify, and validate the data. The results for IHC showed that B cell and neutrophil density was significantly increased during the natural host response in the time period post melanoma cell implantation corresponding to an adaptive immune response (days 7-19) versus immunosusveillance (days 0-6) (p < 0.01, p < 0.001). The density was further increased with immunotherapy treatment (p < 0.01, p < 0.001). For IF, using in silico analysis, we could identify three distinct clusters of CD4+ T cell populations: intermediate, high, and mixed. The cluster was composed of different combinations of biomarkers of CD4 activity. In summary, we developed a powerful method that utilized tissue cytometry to highlight immune cells and cell clusters.