Mechanisms of Local Inflammation Resolution in Subcutaneous Connective Tissue

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Inflammation is a dynamic response developed throughout evolution to defend the host against pathogens or injury. This phenomenon is accompanied by a resolution process orchestrated by the organism to prevent permanent tissue damage. Evidence obtained in our laboratory suggests that resolution can be generated by local components of the subcutaneous connective tissue (CT). We have conducted ex vivo experiments to test this hypothesis. Preliminary results, suggest that (1) Neutrophils, migration is decreased by CT stretching: Neutrophils migration through CT following a chemotactic gradient was reduced in stretched vs. non-stretched tissue (Figure 1D). (2) Stretching of CT increases tissue resolution (RVD1 production: tissue was stretched vs vivo for 4h and RV01 measured by ELISA, n=4, P = 0.1). The effect of stretching on neutrophils migration could be mediated by the local release of pro-resolver mediators. Alternatively, we have previously shown that fibroblasts increase their size following stretching and thus fibroblasts may act as a physical barrier to delay the neutrophil transit. Cross-talk between fibroblasts and CT resident immune cells enhanced by stretch is an anticipated scenario under current investigation.

Human Germinal Center B Cells Express a Unique Glycosylation Signature Characterized by Poly-N-Acetyllactosaminyl Glycans

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Leukocytes commonly remodel cell surface carbohydrates (glycans) upon activation in order to modulate immunologically significant lectin-glycoprotein interactions. To understand how lectin-glycan interactions might direct surface glycosylation of B cell subsets present in human tonsils during ongoing immune responses. Using lectins of characterized glycan specificity, we found that B cells activated with T cell help (germinal center (GC) B cells) exhibit >2-fold higher poly-N-acetyllactosamine (polyLacNAc) glycans compared to naive, memory, and plasmablast B cell subsets. Moreover, these polyLacNAcs are primarily displayed on CD40+ cells, a receptor tyrosine phosphatase critically involved in B cell subset development and maintenance. Furthermore, we observed that the glycosyltransferase ST3Gal1 is selectively repressed in GC B cells and blocks polyLacNAc synthesis when overexpressed in GC-like B cell lines. Unexpectedly, increased polyLacNAc levels did not confer commensurate binding to polyLacNAc-specific, immunoregulatory galectin-1 or galectin-3, but rather was associated with unchanged or reduced binding, respectively. Accordingly, we propose that GC B cells express a unique carbohydrate profile as a means to regulate GC B cell fate and blocks polyLacNAc synthesis when overexpressed in GC-like B cell lines. Unexpectedly, increased polyLacNAc levels did not confer commensurate binding to polyLacNAc-specific, immunoregulatory galectin-1 or galectin-3, but rather was associated with unchanged or reduced binding, respectively. Accordingly, we propose that GC B cells contribute to the modulating immune response by binding to galectins. Because the vast majority of B cells die by cellular suicide during immune responses, we are investigating whether controlled binding to galectins might determine which B cells ultimately survive to fight infection. By studying the role of carbohydrates in B cell immune responses, we hope to understand how B cells protect from infection or go awry in B cell-mediated diseases, such as lupus and rheumatoid arthritis.

Monoclonal Antibodies Targeting Staphylococcus aureus Capsular Polysaccharides Elicit Protection in a Murine Bacteremia Model

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The capsular polysaccharide (CP) produced by Staphylococcus aureus is a virulence factor that allows the organism to evade uptake and killing by host neutrophils. Passive immunotherapy using CP antibodies could be a novel therapeutic approach to prevent and treat severe staphylococcal infections. Interestingly, both the serotype 5 (CP5) and type 8 (CP8) capsular polysaccharides are opsonic and protect mice from experimental bacteremia provoked by encapsulated staphylococci. In this study, we generated monoclonal antibodies (mAbs) against S. aureus CP5 or CP8. The mAbs mediated CP type-specific opsonophagocytic killing of S. aureus strains, but the CP8 O-acetyl specific mAb was not opsonic for a S. aureus mutant that expresses O-deacetylated CPS. Mice passively immunized with mAbs specific for CP5 or CP8 were protected against infection in a murine model of S. aureus bacteremia. Reference strains of S. aureus and a selection of clinical isolates reacted by colony immunoblot with the CP8 polysaccharide, but not CP5. These mAbs alone or in combination with mAbs targeting other virulence factors, may be a promising therapeutic candidate for treatment of staphylococcal infections.

Clinical Implications: The monoclonal antibodies to S. aureus capsular polysaccharides alone or in combination with monoclonal antibodies targeting other virulence factors, may be a promising therapeutic candidate for treatment of antibiotic resistant staphylococcal aureus infections.

Screening For Skin Homing Program Genes In CD8+ T Cells After Live Viral Immunization

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Previous work has shown that after Vaccinia virus(VACV) infection of skin, antigen specific CD8+ T cells in the lymph node draining the infected site begin to proliferate at 24-48 hours, and by 60 hours, cells can be detected that are restricted to VACV expressed P5. While certain factors have been implicated in the acquisition of a skin homing phenotype, no global unbiased approach has yet been applied to this process. In order to accomplish this, we loaded OT-1 T cells with CFSE, injected them into syngeneic mice, and 24 hours later immunized by skin scarification with VACV-ova. At 60 hours after immunization, we obtained draining lymph nodes that contained sorted cells that had undergone between 0 and 5 cell divisions. RNA was extracted from these cells, and was analyzed by transcriptional profiling. Hierarchical clustering of 325 genes showing more than 1.5-fold changes in expression suggested that robust gene expression changes occurred from P0-P5 cell divisions. Subsequent gene ontology (GO) and gene co-expression network were conducted to identify core regulatory genes associated with skin homing. From these data, we can begin to build a genetic blueprint for acquisition of the skin homing phenotype in activated T cells.

Clinical Implications: A more complete understanding of skin immune system is of great significance, which could facilitate the development of new vaccine strategies for skin infectious diseases as well as novel maecrocopics targeting a variety of skin-associated diseases, such as melanoma.

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Scleroderma is an autoimmune disease characterized by inflammation and fibrosis in skin and internal organs. We utilized a murine Graft-versus-Host Disease model of Scleroderma (sclGvHD), in which lymphocytes are transferred from B10.D2 mice (graft) into MHC-matched, lymphocyte deficient BALB/c-Rag2−/− mice (host, sclGvHD mice). Over the course of 6 weeks, sclGvHD mice develop skin inflammation and fibrosis. Previously, we identified IL13 pathway activation in the skin of sclGvHD mice and scleroderma patients. Accordingly, mice lacking IL4RA, a functional subunit for the IL13 receptor, are protected from sclGvHD. Here, we show that sclGvHD-Il4ra−/− mice display a defect in lymphocyte trafficking, with more activated T-cells in subcutaneous lymph-nodes (sLNs), but fewer cells in lymph, blood and skin compared to control mice. Additionally, Sphingosine 1-phosphate (S1P) kinase 1, the main enzyme responsible of S1P production, was decreased in sLNs and lymphatic endothelial cells (LECs) from sclGvHD-Il4ra−/− mice. Since S1P is the major regulator of lymphocyte egress from sLNs, these data suggest that IL4RA promotes sclGvHD by inducing the S1P production from LECs, driving activated T-cells into the periphery. These experiments highlight a potential novel role for IL4RA in inflammatory diseases, and indicate that impeding lymphocyte trafficking may be an effective therapeutic strategy for scleroderma.

Clinical Implications: The fibrosing disease scleroderma lacks specific treatments. Using a mouse model, we discovered that the cytokine IL13 facilitates trafficking of activated immune cells from lymph nodes to skin where they cause disease. Thus, IL13 is new treatment target for scleroderma.