The 707th Kitasato Medical Society Invitational Academic Lecture Series Abstract

(2018.7.9)

A window into endothelial injury mechanisms revealed by S1PR1 GPCR reporter mice

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Injury of the aortic endothelium occurs frequently due to disturbed flow-induced abnormal shear stress, metabolic abnormalities and hypertension. However, chronic endothelial injury contributes to atherosclerosis and aneurysm. Hemodynamic shear stress response of the endothelium is one of the key stimuli. Pulsatile laminar shear forces promote normal homeostasis, whereas disturbed shear forces which are common in geometrically challenged areas of the vasculature (branch points, lesser curvature), lead to endothelial injury and dysfunction. We recently showed that sphingosine 1-phosphate (S1P) receptor-1 GFP signaling mice show marked activation of β-arrestin-coupled GPCR signaling (Galvani et al. (2015) Science Signaling) at sites of endothelial injury. Given the fact that S1pr1 is an inhibitor of vascular injury and atherosclerosis as shown in the aforementioned study, we sorted GFP+ aortic endothelial cells (injured aortic endothelial cells; IAEC) and compared with GFP- aortic endothelial cells (healthy aortic endothelial cells; HAEC). IAEC and HAEC from normal S1pr1 GFP signaling mice (N = 4－5; age ~ 8－10 weeks) were used for systems level chromatin profiling (ATACseq) and RNA expression (RNAseq). From this dataset, we found enrichment of signal transduction pathways influencing immune cell infiltration, inflammation, endothelial mesenchymal transition (EndMT), lipid uptake, and cell proliferation. Expression of unique transcription factors were modulated, and their binding sites were enriched in the open chromatin of IAEC and HAEC. Open chromatin sites were associated with genes that regulate immune cell infiltration, inflammation, EndMT, lipid uptake, and cell proliferation. These data suggest that either S1pr1 β-arrestin coupling which is associated with GPCR endocytosis and biased signaling regulates transcriptional events involved in endothelial injury. Alternatively only some of these genes are regulated by S1pr1 signaling, whereas the others are regulated directly by disturbed flow-induced biomechanical signaling. We will present our recent data in this area of investigation. These studies are expected to further increase our understanding of endothelial injury at the molecular level and may ultimately lead to the development of novel approaches to treat endothelial injury which occurs in many diseases, including chronic inflammatory, autoimmune, cardiovascular, neurological and metabolic conditions.

The 708th Kitasato Medical Society Invitational Academic Lecture Series Abstract

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Innate lymphoid cells (ILC2) and surfactant protein D (SP-D) in allergen and air pollution-induced airway inflammation

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Asthma and COPD are characterized by chronic airway inflammation, airway hyperresponsiveness (AHR) and airway remodeling. Our group demonstrated the protective importance of the epithelial derived molecule surfactant protein-D (SP-D). We also identified a pathogenic significance of the pro-inflammatory group 2 innate lymphoid cells (ILC2) in airway inflammation, brought on by exposure to allergen and the toxic air pollutant, ozone. But the relationship between SP-D and ILC2 in the lung and the mechanisms through which ILC2s promote AHR and SP-D interferes with this function, have not been investigated before.

To study the hypothesis that SP-D suppresses ILC2 activation, wild type and SP-D-/- mice were exposed to ozone (O3) for 2 hours and investigated 12 hours later. In the absence of SP-D ILC2 activation was enhanced and these cells also produced IL-17A, a pro-neutrophilic mediator. This was a surprising finding because IL-17A is a cytokine previously thought to be exclusively generated by Th17 cells and another subtype of innate lymphoid cells, ILC3. Our results suggested that ILC2 has cellular plasticity that can contribute to neutrophilia. Consequently, SP-D-/- mice had heightened and prolonged airway neutrophilia after O3 exposure, that corresponded with significantly increased ILC2 counts. O3 induced ILC2 activating cytokines in the lung of both wild type and SP-D-/- mice but lack of SP-D was associated with increased lung IL-33 mRNA expression, elevated ILC2 counts and higher ST2 (IL-33 receptor) GATA-3, Bcl11b and lower RORγT expression by ILC2. Further, in response to ozone inhalation, ILC2s acquired a hybrid functional phenotype associated with simultaneous ILC2 and ILC3 cytokine profile. We propose, therefore, that in the absence of SP-D, ILC2 are "primed" for inflammatory changes including phenotypic alterations. In contrast, recombinant SP-D directly suppressed activation of these cells in a dose dependent manner *in vitro*. Patients with severe asthma, when compared with healthy subjects also had increased numbers of circulating ILC2 that expressed a proinflammatory mixed ILC2/ILC3 cytokine profile supporting the pathogenic role of these cells in severe airway inflammation.

In addition to a heightened inflammation, ILC2 activation was also associated with airway hyperresponsiveness in mouse models of allergen and ozone exposure. Our next questions were, what kinds of inflammatory molecules are produced by ILC2s to generate this airway change? How do these innate lymphoid cells cause airway obstruction? To answer these, we studied a mouse model of allergen (Alternaria alternate) exposure. Within hours, immune cells had infiltrated the airways, generating inflammation and vascular endothelial growth factor A, (VEGFA, formerly VEGF) was one of the highest expressed genes in activated ILC2. VEGFA was also very strongly upregulated in ILC2s isolated from the peripheral blood of asthma patients. This mediator can actually, by itself, elicit the symptoms of asthma in mice. Indeed, when the mice were treated with a VEGFA inhibitor SU1498, the allergen-induced AHR declined. Further study showed that ILC2s were stimulated by interleukin-33, (IL-33), another pro-inflammatory immune molecule, which also enhanced AHR and increased VEGFA's cellular receptor, VEGFR2. Treatment with SU1498 mitigated all these responses, too, indicating that VEGFA mediates the ILC2 effects on AHR.

In conclusion, in mouse models and severe asthma patients, we demonstrated that lung resident innate lymphoid cells have a prominent pathogenic role in both airway inflammation and AHR and that SP-D plays an immunoprotective function in the lung by directly suppressing pro-inflammatory activation and phenotypic alterations of these cells.

The 709th Kitasato Medical Society Invitational Academic Lecture Series Abstract

(2018.7.9)

Regenerative lymphangiogenesis with gastrointestinal tract

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In the past dozen years, an expanded repertoire of genes and molecular pathways involved in the development of the lymphatic vascular system has been elucidated. However, considering the essential role of lymphatic vessels in intestinal lipid absorption and the increased prevalence of inflammatory diseases of the intestine, it is rather remarkable that there are currently more questions than answers regarding whether and/or how lymphatic vessels contribute to (or may be causative of) pathophysiological diseases in adults. Our research group directly addresses many of these questions by building upon our exciting discoveries on the essential roles of adrenomedullin (AM) signaling in lymphatics. For example, our recent studies have used an inducible knockout allele to show that loss of the adrenomedullin receptor in adult animals fully recapitulates the clinical sequelae related to lymphangiectasia, including dilated lymphatics, reduced intestinal lipid absorption, protein losing enteropathy, and limb edema. Current studies build upon these exciting findings and strive to elucidate the physiological and molecular processes that lymphatics play in i) intestinal disease initiation and progression, ii) normal intestinal lipid absorption under a variety of different challenging conditions and iii) the initiation and progression of mucosal injury, inflammation, and repair. The elucidation of these molecular pathways may ultimately form the basis of GPCR-targeted approaches for the therapeutic modulation of intestinal lymphatic vessels, particularly during lymphangiectasia and disease conditions associated with digestive tract inflammation.

The 710th Kitasato Medical Society Invitational Academic Lecture Series Abstract

(2018.7.9)

Molecular clocks and the human chronobiome

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Molecular clockworks are tightly controlled with a high degree of redundancy. As a highly conserved system it serves to link biological networks across tissues and its disruption results in metabolic and inflammatory phenotypes that reflect a loss of such consolidation. Molecular clocks also contribute to the interactions between the host and the gut microbiome. Loss of the integrative role of clockworks is apparent in aging and has been linked to neurodegenerative syndromes and to cancer.

Interactions between clockworks and such integrative phenotypes is classically bi-directional and discrimination of cause and effect is challenging. Many of our insights into clock function depend on deletion of Bmal-1, the only non-redundant core clock gene in mice. Conventional deletion strategies result in many hallmarks of reduced aging including a reduction of lifespan by ~2/3. However, postnatal deletion of this gene results in mice with a normal lifespan and some "aging" phenotypes are lost or flipped. This may reflect "off target" actions of Bmal-1 during development. However, neurodegenerative and ocular phenotypes are conserved, and use of both reagents converge on a clock-dependent role of Bal-1 in neuroprotection.

We know little about the role of the clock in human aging although loss of oscillatory phenotypes are observed and a loss of consolidation of the sleep-wakefulness cycle predicts dementia. We have recently wished to characterize the human chronobiome, combining remote sensing and multi-omics approaches. Here, we seek subsets of data where time of sampling supercedes interindividual variability as a contributor to variance in humans "in the wild." As we go to scale, with the basal and evoked chonobiome, we plan to determine whether erosion of the oscillatory phenotypes might predict patterns of aging.

The 711th Kitasato Medical Society Invitational Academic Lecture Series Abstract

(2018.7.24)

Innate-like lymphocytes in health and disease

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The immune system contains subsets of lymphocytes that cannot be easily classified as either innate or adaptive, and these cells are instead called, innate-like lymphocytes. Such lymphocytes express antigen-specific receptors characteristic of the adaptive immune system, yet their effector functions are more similar to cells of the innate immune system. Our lab focuses on two subsets of innate-like lymphocytes: invariant natural killer T (iNKT) cells and regulatory B cells (Bregs). iNKT cells are a group of innate-like T cells that express a semi-invariant T cell receptor specific for lipid antigens presented by the CD1d protein. Upon activation, iNKT cells rapidly secrete multiple cytokines and potently modulate responses mediated by other immune cells. Such immunoregulatory functions of iNKT cells have been exploited for immunotherapeutic purposes. Many of these studies have been performed with the prototypical iNKT cell antigen α-galactosylceramide (α-GalCer). Our lab has made important contributions to our understanding of iNKT cell function in health and disease, and their therapeutic potential in a variety of autoimmune and inflammatory diseases. Another group of innate-like lymphocytes is Bregs cells that are comprised of a number of distinct subsets. These cells are present in multiple anatomic locations, including the spleen, peripheral blood, and the peritoneal cavity, and modulate immune and inflammatory responses by producing anti-inflammatory mediators such as IL-10. We have found that these cells are present in mouse white adipose tissue (WAT), and that WAT expansion during obesity progressively depletes Bregs. We have further observed that the spleen supports a functional reservoir of Bregs for WAT infiltration and affords protection against obesity-induced insulin resistance in mice. Our current studies focus on the contribution of Bregs to cardiovascular disease, using experimental models of myocardial infarction. Collectively, our studies have identified innate-like lymphocytes as novel targets for a variety of inflammatory diseases.

The 712th Kitasato Medical Society Invitational Academic Lecture Series Abstract

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Pediatric heart transplantation in the US

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Experience of pediatric heart transplantation in Japan has been accumulating, but is still very limited with only three centers across Japan where pediatric heart transplantation can be performed. In the US, pediatric heart transplantation is an established therapy for patients with end-stage heart failure.

In my lecture, system and actual steps of pediatric heart transplantation in the US will be presented. Surgical procedures for simple and complex heart transplantation will also be described based on the author's experience.