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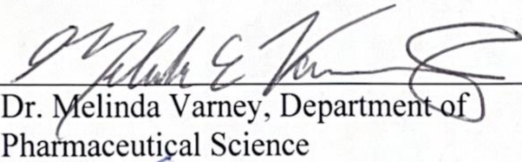
ELUCIDATING THE IMPACT OF WESTERN DIET ON BONE MARROW CYTOKINE SIGNATURES: UNDERSTANDING POOR OUTCOMES IN SUSCEPTIBILITY TO INFECTION AND PROGRESSION OF CANCER

A thesis submitted to
Marshall University
in partial fulfillment of
the requirements for the degree of
Master of Science
in
Pharmaceutical Sciences
by
Lahari Kondeti
Approved by
Dr. Melinda E Varney, Committee Chairperson
Dr. Jeremy McAleer, Committee Member
Dr. Cynthia B Jones, Committee Member

Marshall University
May 2023

Approval of Thesis

We, the faculty supervising the work of Lahari Kondeti, affirm that the thesis, *Elucidating the Impact of Western Diet on Bone Marrow Cytokine Signatures: Understanding poor outcomes in susceptibility to infection and progression of cancer*, meets the high academic standards for original scholarship and creative work established by the Department of Pharmaceutical Science and the Marshall University School of Pharmacy. The work also conforms to the requirements and formatting guidelines of Marshall University. With our signatures, we approve the manuscript for publication.



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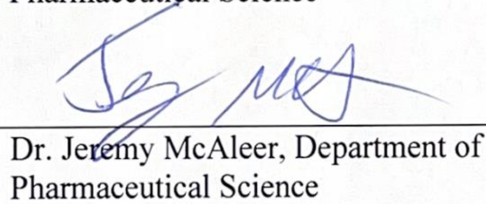

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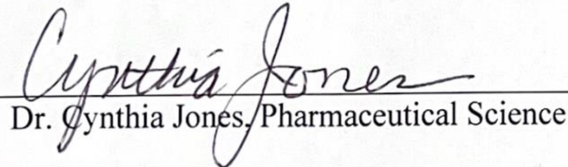

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Abstract

Among other disease outcomes, obesity is associated with 1) reduction in long-lasting immune protection and 2) acquisition of cancer. Given our interest in hematopoietic stem and progenitor cell (HSPC) dynamics, we questioned if similar mechanisms in the bone marrow microenvironment contribute to obesity-induced HSPC alterations responsible for 1) decreased immune protection and 2) transformation of HSPCs in the pathogenesis of hematologic malignancies. The negative effects of obesity on immune protection are most notably established in influenza models. Influenza infection susceptibility is increased and influenza vaccine-induced immunity wains in obese individuals. Ongoing studies aim to define mechanisms for waning immunity at sites of infection. Little is understood, however, regarding how obesity affects HSPC dynamics in the bone marrow microenvironment upon immunization. We hypothesized that obesity alters the types and quantities of immune cells produced by HSPCs upon immunization. We further hypothesized that this is due to altered availability of cytokines present in the bone marrow. Similarly, we hypothesized that unique cytokine signatures in the bone marrow of obese individuals serve as a mechanism for cancer initiation and progression in those with genetic susceptibility to MDS. To test our hypotheses, we subjected applicable mouse models to low-fat or high-fat diets. In our immunization studies, results indicate that high-fat diet leads to myeloid skewing in flu-immunized mice and that cytokine trends in the bone marrow provide insight into how this occurs. In our cancer studies, MDS-susceptible mice fed a high-fat diet displayed distinct cytokine signatures when compared to those fed a low-fat diet, suggesting that this may be a mechanism by which obesity contributes to hematologic malignancy. Further understanding of the contribution of obesity-induced bone marrow cytokine signatures to poor

health outcomes may inform the design of future vaccine formulations or MDS therapeutics for obese patients.

Chapter 1: Introduction

Hematopoiesis

Bone marrow is composed of different types of cells. Hematopoietic stem and progenitor cells (HSPCs) are early cells which have the capacity to self-renew and also differentiate to produce short-term hematopoietic stem cells (HSCs) or multipotent progenitors (MPPs). MPPs, in turn, differentiate and become common lymphoid and myeloid progenitor cells. Common myeloid progenitors differentiate to become more specialized immune cells like megakaryocytes, erythrocytes, mast cells, myeloblasts. Myeloblast is a premature cell of leukocyte family. Myeloblasts eventually mature to become basophils, eosinophils, neutrophils, or monocytes. Monocytes enter tissues and specialize further to become type 1 or type 2 macrophages. Common lymphoid progenitor cells differentiate into natural killer cells, B lymphocytes, or T lymphocytes (Rasheed, 2022).

Components of the Bone Marrow Microenvironment

The bone marrow microenvironment consists of a cellular compartment, liquid compartment, and extracellular matrix. The cellular compartment consists of hematopoietic cells and non-hematopoietic cells. Hematopoietic cells include those cells discussed in the previous section. Non-hematopoietic cells include osteoblasts, osteoclasts, adipocytes, fibroblasts, endothelial cells and effector immune cells. The liquid compartment has mixture of cytokines, chemokines, and growth factors (Mayani et al., 1992).

Obesity

Rates of obesity have been steadily increasing over time. Obesity is one of the crucial factors in poor health outcomes. It increases the risk of deadly and debilitating diseases which include diabetes, heart disease, and cancer. People with a body mass index (BMI) higher than 35 are the most prone to type 2 diabetes (Maggio & Pi-Sunyer, 2003). Body weight is also directly

correlated with risk of cardiovascular diseases. Increases in BMI are attributed to increases in blood pressure, triglycerides, cholesterol, and inflammation. This can lead to shock or cardiovascular death (Powell-Wiley et al., 2021). Obesity is a contributor or risk factor in many types of cancer (Tumminia et al., 2019). Obesity leads to inflammation, which may also contribute to insulin resistance. Because of insulin resistance, cells produce more and more insulin which triggers the proliferation of cells. If proliferation have unchecked DNA damage, this can lead to cancer initiation and progression. Increase in insulin also elevates estrogen, (De Paoli et al., 2021) which is also responsible for cancer. Obesity also leads to increased susceptibility to infection for select pathogens. Regarding respiratory pathogens, obesity can also impair respiratory function by deposition of excess fat in air way organs (Avgerinos et al., 2019). Beyond infection susceptibility, obesity in some instances contributes to more rapid waning of immunity following naïve infection or immunization.

Obesity and Inflammation

Obesity is a major public health concern. It has increased among the U.S. population over the past 30 years based on measured heights and weights, which are utilized to calculate body mass index (BMI). According to World Health Organization reports, at least 1 billion adults are obese, and the numbers are expected to increase in the future if there is no interruption (Marshall, 2004). Most research suggests that key contributor of obesity to disease state is obesity induction of chronic, low-grade inflammation. Due to excess energy intake, the metabolic signals from adipocytes, which are the cells that make up adipose tissue, trigger the inflammatory program and damage metabolic homeostasis (Hotamisligil et al., 1993). The first cytokine discovered to be largely expressed in adipocytes was tumor necrosis factor alpha (TNF-alpha). TNF-alpha expression leads to inflammation processes. It was found that obese individuals express 2.5 times

more TNF-alpha mRNA transcripts in adipose tissue compared to lean controls. TNF-alpha over-expression is established to contribute to resistance to insulin in the body (Hotamisligil et al., 1995)). Along with TNF-alpha, several other cytokines and bioactive substances are produced from the site of adipose (Shoelson et al., 2006).

Indeed, IL-6 is released excessively from omental adipose tissue compared to subcutaneous adipose tissue. The omentum refers to a layer of connective-tissue structure that contains arteries, veins, lymphatics, and fat pads. One of the known functions of IL-6 is the downregulation of lipoprotein lipase in adipose tissue (Fried et al., 1998). Since lipoprotein lipase is essential in lipid homeostasis and functions in breaking down fat (Zechner, 2000), excess IL-6 induced by inflammatory processes contributes indirectly to loss of fat breakdown in obese individuals.

Resistin, a polypeptide that is secreted from adipocytes, is largely found in white adipose tissue when compared to brown adipose tissue. White adipose tissue stores triglycerides while brown adipose tissue is involved in the transfer of energy from food to the production of heat. Obese individuals have less brown adipose tissue than those with a healthy weight. In one experiment, mice fed a high-fat diet have shown increased resistin levels in their serum within four weeks of consuming a high-fat diet (Steppan et al., 2001). Resistin leads to target tissue insulin resistance, and it has created a link between obesity and insulin resistance (Steppan et al., 2001). Conversely, an adipocytokine called visfatin, which is secreted mostly in visceral fat, mimics the functions of insulin (Fukuhara et al., 2005). Interestingly, visfatin is structurally identical to the pre-B cell colony enhancing factor (PBEF). PBEF accelerates the maturation of B cell precursors. PBEF and therefore visfatin is proinflammatory and has been shown to promote TNF-α, interleukin (IL)-1, IL-16, transforming growth factor (TGF)-β1, and the chemokine receptor CCR3. PBEF upregulates IL-6, TNF-alpha and IL-1 production in many immune cells. Increased levels of

visfatin are associated with increasing levels of IL-8, C-reactive protein and monocyte chemotactic protein-1 (MCP-1). Visfatin also plays a key role in the persistence of inflammation by inhibiting neutrophil apoptosis. While visfatin is associated with insulin-like function, alterations in levels of visfatin during obesity may indicate an attempt to regulate blood glucose levels to keep them stable. When visfatin levels rise beyond a threshold, however, inflammatory processes persist, contributing to the development of insulin-resistance, diabetes, cardiovascular disease, and renal disease (Mona Mohamed Ibrahim Abdalla, 2022). In addition to resistin and visfatin, plasminogen activator inhibitor-1 (PAI-1) RNA is known to increase with obesity, specifically in visceral fat, which may lead to vascular disease in visceral obesity (Shimomura et al., 1996).

The kinases, namely c-Jun amino-terminal kinases (JNKs), inhibitor of k kinase (IKKB), and protein kinase R(PKR) play important roles in transmitting metabolic signals, whether it is extracellular or intracellular. These kinases have a potent function in innate immune signaling. It was shown that JNK activity was increased in response to obesity. A decrease in JNK1, remarkably increased insulin sensitivity to the cells. JNK and IKK act as mediators in the inflammatory pathway that elevates the expression of pro-inflammatory cytokines and chemokines (Hirosumi et al., 2002), (Solinas & Karin, 2010).

Another feature of the inflammatory phase of obesity is the infiltration of immune cells in adipose tissue. Macrophages, mast cells, natural killer cells, and T cells have shown an increase in number in obese adipose tissue. (Gregor & Hotamisligil, 2011). Mast cells secrete IL-6 and MCP-1, which are pro-inflammatory cytokines that lead to inflammation in adipose tissue (Michailidou et al., 2022). Lipids such as palmitate and other unsaturated fatty acids can bind to toll-like receptors (TLRs) on the surface of immune cells, such as macrophages, and are converted to ceramides and diacylglycerols during states of lipid overabundance as occurs in

obesity. These toxic lipids enhance proinflammatory signaling. Macrophages present in these adipose tissues upregulate cytokines such as TNF and IL-6 which in turn promote mast cell accumulation. As natural killer (NK) cells are activated in the innate immune pathway by IL-15. IL-15 presence in adipose tissues triggers NK cell proliferation leading to inflammation in adipose tissues. CD8+ T cells also promote macrophage infiltration in the adipose tissue during obesity, mainly through the secretion of factors that induce macrophage migration, such as MCP-1 and MCP-3 (Michailidou et al., 2022).

Obesity Impact on Hematopoietic Stem and Progenitor Cells

Given that inflammatory and other cell signals from adipose tissue impact the composition of the bone marrow microenvironment, there is a need to study obesity and HSPC dynamics. There is some controversy that exists in understanding the HSPCs in obesity. Many researchers have claimed reduction in HSPCs, while others claim that they increase in number. This may have to do with the timing of studies. HSPCs may be so expansive in chronic inflammatory states that it leads to stem cell exhaustion. The scientific community has not come to a conclusion concerning HSPCs dynamics in obesity (Benova & Tencerova, 2020). From a previous study, it was shown that HSPCs have decreased proliferation potential in the bone marrow of obese mice. In the study, C57BL/6 mice were fed with high-fat diet (45% kcal from fat) up to 18 weeks. Researchers showed that there was differentiation potential in HSPCs, however there was a decrease in proliferating capacity of HSPCs in obese mice bone marrow, ultimately leading to disturbances in production of mature immune cells (van den Berg et al., 2016) (Naveiras et al., 2009). On the other hand, some researchers have found that obesity promotes expansion of myeloid-skewed HSPCs. One example is a study from Nagareddy et al. who demonstrate that there is increase in expansion, differentiation and proliferation of HSPCs in obese mice bone marrow (Nagareddy et al., 2014).

Many factors may contribute to the differences in data acquired in various studies. Diet-induced obesity is indeed multifactorial in nature. Consequences could depend on genetics (Habanjar et al., 2022), epigenetics, and environmental exposures. In the current literature, experiments vary in using several different genetic models, experiment diets that differ in the form of the fat in the calories, varied length of the exposure to the diets, and various sources of mice which impact their microbiome.

Obesity Impact on the Bone Marrow Microenvironment

Bone marrow has a heterogeneous niche with different immune cell types. HSPCs interact with these cells and osteoblasts, osteoclasts, endothelial cells, mesenchymal cells, and adipocytes. Obesity plays a crucial role in accumulating several adipocytes in the bone marrow. As the long-term consequence of obesity, the number and size of adipocytes taking the space in bone marrow increases, which contributes to disturbed HSPC interaction with neighboring cells. This ultimately leads to uncertain immunity. Osteoblast activity changes with obesity, and this ultimately influences HSPCs (Adler, Kaushansky, et al., 2014). Mesenchymal stem cells (MSC) exist in the bone marrow microenvironment with close proximity to HSPCs. MSCs regulate HSPC populations via a chemokine, CXCL12 (Greenbaum et al., 2013). Disruption of bone marrow morphology with adipogenesis makes it difficult for MSCs and osteoblasts to maintain HSPCs. There is also a decrease in more specialized immune cells in bone marrow in case of obesity (Nedunchezhiyan, 2022). In one study, mice were fed with high-fat (45% kcal in fat) diet for 12 weeks. B cells and T cells were analyzed through flow cytometry. It was found that compared to regular diet there was a decrease in proportions of B cells and T cells in both the bone marrow and peripheral blood in mice fed with high-fat diet (Chan et al., 2012), (Adler, Green, et al., 2014). Adiposity prevents generation of pro-B cells in rabbits. This was tested by adding adipocyte conditioned medium of rabbits along with OP9 mouse stromal cells and bone marrow mononuclear cells from rabbits of greater than eight weeks of age.

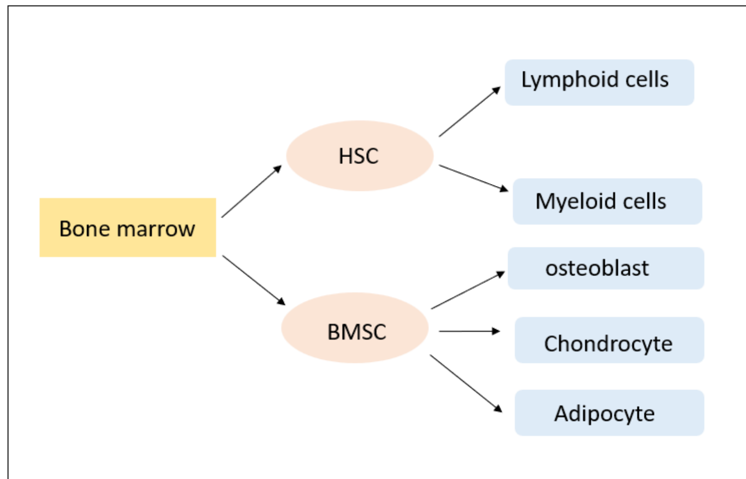
Bilwani et al. found that CD79a+ cells were not spawned in the presence of adipocyte conditioned medium in this system that mimics bone marrow interaction with stromal cells. CD79a+ is a marker for B cells. Therefore, it suggests that B cell lymphopoiesis is inhibited in presence of adipocytes (Bilwani & Knight, 2012).

Bone marrow also acts as an endocrine organ where it secretes the hormones. Obesity tends to affect the bone marrow, which in turn affects the production of hormones. Hormones are produced from various cells that are located in the bone marrow. Some of the cells include lymphocytes and plasma cells. The role of lymphocytes is to improve the adaptive immunity of the body. Researchers have found that the bone marrow cells comprise of 8% of the lymphocytes and 1% of the plasma cells (Hou et al., 2020). Some of the lymphocytes include T cells and B cells. Importantly, the natural killer cells are also located in the bone marrow and function to offer protection. Megakaryocytes in the bone marrow ensure that platelet production continues. This is achieved by producing a hormone known as thrombopoietin.

Bone marrow not only houses cells after they are developed and prior to their mobilization, but it also acts to store other cells for the lifetime of the cell, such as neutrophils which protect the body. In conclusion, the bone marrow cells attempt to ensure homeostasis (Suresh et al., 2020), but obesity tends to affect the microenvironment and may throw everyday processes off track (Suresh et al., 2020). Figure 1.1 below shows a simple schematic of the cells found in the bone marrow.

Figure 1.1

Composition of Bone Marrow



Note. HSC: Hematopoietic stem cells, BMSC: Bone marrow mesenchymal stem cells. HSCs give rise to lymphoid cells and myeloid cells. BMSCs give rise to osteoblasts, chondrocytes, and adipocytes.

Moreover, obesity tends to affect the microenvironment of the bone marrow in the production of the red blood cells. Bone marrow produces hormones, including the hormone erythropoietin, which increases the production of red blood cells. Obesity affects the production of red blood cells and turns the red bone marrow into yellow bone marrow (Tencerova et al., 2019). Yellow bone marrow is low in erythropoietin, thus will tend to reduce the production of red blood cells and ultimately lead to anemia.

The bone marrow microenvironment has complex signaling pathways which are mediated by transcription factors. These factors regulate the differentiation and proliferation of HSPCs and bone marrow MSCs. The table below shows the key factors, their function on type of cell and their alteration in case of obesity.

Table 1.1

Function of Transcription Factor in Bone Marrow Microenvironment

Intrinsic Regulators	Cell Type	Function	Impact of Obesity on Transcription Factor
GATA1-3 (erythroid transcription factor)	HSC	Transcription factor regulating HSC lineage determination	↑GATA3
Gfi 1 (Growth factor independent 1 transcriptional repressor)	HSC	Transcription factor regulating HSC quiescence and self-renewal	↑
PPARγ (Peroxisome proliferated activated receptor gamma)	MSC	Regulates adipogenesis	↑
TGF-β1(Transforming growth factor)	MSC	Negative regulator of adipogenesis	↓
BMP-2(Bone morphogenic protein)	MSC	Positive regulator of osteoblast differentiation	↓

Note. HSC- Hematopoietic stem cell; MSC- Mesenchymal stem cells (Benova & Tencerova, 2020).

Obesity also affects the microenvironment of the bone marrow by altering actions of the bone marrow MSCs. Bone marrow MSCs are known to contain immunosuppressive properties which ensure that there is regulation of the proliferation process in the bone marrow. Indeed, bone marrow MSCs have the human leucocyte class 1 and CD (80, 86 and 40) which regulate the proliferation of the T cells (Reagan et al., 2021). Therefore, the bone marrow MSCs are involved in regulating various other immune functions. Obesity tends to influence these functions. Importantly, the role of the bone marrow MSCs depends on several factors including immunity and developmental factors. It is worth noting to understand that bone marrow MSCs have several

secretory factors that aid in the homeostasis of the bone marrow. These are discussed in the next section.

Obesity Effects on Bone Marrow Cytokines

The bone marrow MSCs secrete several secretory factors called cytokines. These factors regulate the cell-to-cell interactions and immunoregulatory functions of MSCs. The table below shows the various secretory factors and how they are modulated by obesity (Benova & Tencerova, 2020).

Table 1.2

Various Secretory Factors by BMSCs

Secretory Factor	Function	Impact of Obesity on Cytokine
M-CSF	Myelopoiesis	=
PGE2	Inhibition of T-cell proliferation	↓
HGF	Inhibition of T-cell proliferation	-
TGF	Inhibition of T-cell proliferation	↓
IL-7	B cell development	↓
IL-15	T cell homeostasis	↓
IL-21	NKT cells maturation	-
TNF-alpha	HSC proliferation and activation	↓, ↑
SDF-1	Stem cell migration	↓
Thrombopoietin	HSC quiescence	↑
Angiopoietin	HSC quiescence	↑
G-CSF	Myelopoiesis	↑
RANKL	Osteoclast differentiation	↑

Aside from cytokines secreted by bone marrow MSCs, others are altered in circulation. IL-1beta, Leptin, TNF alpha, IL-6, MCP-1 are all increased in circulation due to obesity. IL-6 and TNF alpha have increased osteoclastic activity which leads to bone breakdown. IL-1 beta and MCP-1 have also been shown to have effect on osteoblastogenesis in an inhibitory manner. Specifically in bone marrow there is an increase in IL-1 beta, TNF alpha, IL-6 in mice bone marrow isolates. These pro-inflammatory cytokines contribute to bone marrow niche remodeling due to marrow adipose tissue (Emmons et al., 2017). They induce hematopoiesis in bone marrow, increase the differentiation of myeloid cells and accelerates the proliferation. This differentiation is enhanced due to increases in cell cycle activators, decreases in cell cycle inhibitors, and also augmentation of myeloid lineage genes. TNF-α functions in a dose dependent manner with regard to hematopoietic stem cells and also depends on the type of the progenitor cells. In some studies, it was shown that TNF-α reduces the differentiation of hematopoietic stem cells, on the other hand it was known to have negative effects on HSCs. (Selleri et al., 1995) (Yamashita & Passegue, 2019). From the previous studies, it was shown that human bone marrow adipocytes are the source of several cytokines like LIF, CCL3, CSF3, CCL4, CCL2, IL-23A, IL-6, CXCL10, CXCL2, PF4, CXCL1, and IL-1β rather than adipose tissue adipocytes or bone marrow mesenchymal cells (Mattiucci et al., 2018). The functions of these cytokines are summarized in the table below. Murine bone marrow adipose tissue shows that adipocytes and leptin receptor stromal cells secrete stem cell factor (SCF) which promotes hematopoietic regeneration (Zhou et al., 2017).

Table 1.3

Cytokines Secreted by Bone Marrow Adipose Tissue and Their Functions

Cytokine	Function	References
LIF (Leukemia inhibitory factor)	inducing differentiation of leukemia cell, inflammatory response, stem cell self-renewal	(Yue et al., 2015)
CCL3 (Chemokine (C-C motif) ligand 3)	involved in the acute inflammatory state in the recruitment and activation of polymorphonuclear leukocytes through binding to the receptors CCR1, CCR4 and CCR5	(Sherry et al., 1988)
CSF3 (colony-stimulating factor 3)	stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream	(Deotare et al., 2015; Tay et al., 2017)
CCL4 (Chemokine (C-C motif) ligands 4)	contributes to inflammation by recruiting other leukocytes at site(s) of inflammation	(Sindhu et al., 2019)
CCL2 (chemokine (C-C motif) ligand 2)	regulates cellular mechanics and thereby recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation.	(Evers et al., 2022), (Carr et al., 1994), (Xu et al., 1996)
IL-23A (Interleukin-23 subunit alpha)	it promotes upregulation of the matrix metalloprotease MMP9, increases angiogenesis and reduces CD8+ T-cell infiltration	(Memari et al., 2015)
IL-6 (Interleukin 6)	stimulation of acute phase responses, hematopoiesis, and immune reactions	(Tanaka et al., 2014)
CXCL10 (C–X–C motif chemokine 10)	induces chemotaxis, apoptosis, cell growth inhibition	(Liu et al., 2011)
CXCL2 (Chemokine (C-X-C motif) ligand 2)	neutrophil chemoattractant and is involved in many immune responses.	(Al-Alwan et al., 2013)
PF4 (Platelet factor 4)	promote blood coagulation, and is involved in innate and adaptive immunity	(Cai et al., 2020)
CXCL1 (chemokine (C-X-C motif) ligand 1)	activates phosphatidylinositol-4,5-bisphosphate 3-kinase-γ (PI3Kγ)/Akt, MAP kinases such as ERK1/ERK2 or phospholipase-β (PLCβ) signaling pathway.	(Silva et al., 2017),(Devalaraja et al., 2000)
IL-1β (interleukin-1β)	An important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis.	(Lopez-Castejon & Brough, 2011)

Vaccines

Arguably one of the world's most important scientific achievements, vaccines are biological preparations of pathogens or isolated proteins of pathogens, which provide us with active acquired immunity. Priorities in vaccinations include ensuring that they are safe and effective in protecting people against future exposures to harmful pathogens. Most vaccines consist of inactive parts of a particular organism. This then which triggers immune response in the recipient of the vaccine. Having inactive organisms or pieces of organisms in composition of vaccine ensures that it will not cause harm or actively replicate in the body. The goal of vaccination is to provide lasting immune memory, with relies heavily on the production of antibodies (Maltezou et al., 2019).

Vaccine Impact on Hematopoietic Stem and Progenitor Cells

Impact of immunization on HSPCs is a newer approach to the development of a vaccines. Varney et al. discovered that *Bordetella pertussis* vaccine content impacts HSPC expansions and B cell differentiation dynamics. *Bordetella pertussis* whole cell vaccine stimulates expansion of MPPs and also attributes to the rapid maturation of B cells upon infection, through HSPC priming. They also found that this is to transcriptional alterations that suggest that interferon (INF) gamma plays a major role in these effects on HSPC dynamics (Varney et al., 2018). Immunization has been established to result in the mobilization of the immune cells to the peripheral bloodstream from bone marrow. This has been achieved by transcriptional changes in bone marrow niche and in some cases by upregulation of cytokines, which actually drive immune cells in the bone marrow to the site of infection. One example is the bacille Calmette-Guerin (BCG) vaccine which is a strain of attenuated form of *mycobacterium bovis*. It causes the HSPC lineage-Sca1+ckit+ (LSK) population in bone marrow to expand (Kaufmann et al., 2018). On further investigating the effect of BCG on HSCs by performing

RNA sequencing, Kaufmann et al. found differentially expressed genes in HSCs and MPPs in BCG intravenously vaccinated mice bone marrow. The transcriptomic changes led HSCs to differentiate to MPPs. Additionally, they have found that MPPs were more polarized towards the myeloid lineage. Similar to the discoveries of Varney et al., intravenous BCG vaccine leads to upregulation of interferon dependent gene expression that in turn causes expansion of the LSK population in bone marrow (Kaufmann et al., 2018).

Myelodysplastic Syndromes

Myelodysplastic syndrome (MDS) is a group of disorders that is characterized by abnormal development of blood cells in the bone marrow. Del5q MDS is one type of MDS which occurs due to chromosomal deletions in particular regions (commonly deleted regions) of chromosome 5q. Deletion of genes within these regions leads to abnormal or dysregulated signaling primarily in immune-related pathways (Varney, Melgar, et al., 2015) (Sallman & List, 2019). MDS in general are heterogenous clonal HSPC disorders, and they are characterized by ineffective hematopoiesis. MDS will occur when blood cells in bone marrow are poorly formed (Tehranchi et al., 2010). MDS are classified based on how the cells look under the microscope and the genetic profile of bone marrow cells. Some classification of are MDS with single-lineage dysplasia (MDS-SLD), MDS with multilineage dysplasia (MDS- MLD), MDS with ring sideroblasts (MD-RS), MDS with isolated del(5q), and MDS with excess blasts (MDS-EB) (Zhang et al., 2022).

Bone Marrow Microenvironment in Myelodysplastic Syndromes

In MDS, the bone marrow microenvironment is dysregulated leading to abnormal growth and differentiation of cells. This dysregulation can be attributed to genetic mutations in the bone marrow cells themselves, as well as alterations in surrounding stromal cells and immune cells. In

MDS, there is disruption of most of the hematopoietic lineages in bone marrow such as myelopoiesis, erythropoiesis, and megakaryopoiesis. There are areas of edema, disrupted sinusoids, fibrosis, lymphoid aggregates, increased number of plasma cells, mast cells, and at times lymphocytes in bone marrow. There is also an imbalance in cytokines of bone marrow, with an increase of TNF alpha, IL-1 beta, IL-6, IL-8, TGF-beta causing hematopoietic cell death in MDS. Hematopoietic stem cell quiescence is maintained by cytokines like stem cell factor, thrombopoietin and angiopoietin-1, aberrant expression of these cytokines are found in MDS, which leads to dysregulated proliferation or depletion of HSC (Raaijmakers, 2012).

Mesenchymal stem cells (MSCs) in bone marrow form the strongest support system for hematopoietic stem cell maintenance. MSCs are source of cytokines like CXCL12, angiopoietin 1, KIT ligand (SCF) which helps in regulation of HSPCs. In MDS, mesenchymal-osteolineage cells are disrupted leading to HSC dysfunction. (Clinckaert et al., 2022). From the previous study, impaired growth of mesenchymal stromal cells is seen in MDS patients, leading to cellular senescence(Geyh et al., 2013).

Statement of the Problem

From the above discussion, it is clear that several studies have been conducted concerning the effects of obesity on hematopoiesis. However, few studies have focused on the effect of obesity on HSPCs in immunization or MDS. Therefore, the results of our studies will not only bridge a gap in existing knowledge but will also contribute to the scientific community by providing knowledge on the effects of obesity as a contributor to poor health outcomes.

Objective: Elucidate the Role of Diet-Induced Obesity in Altering Bone Marrow Cytokine Signatures in Health and Disease.

Specific Aims:

Aim 1) To determine the extent to which obesity impacts bone marrow cytokine signatures and HSPC dynamics following influenza immunization. Our hypothesis is that obesity induces alterations in 1) bone marrow cytokine signatures and 2) the types and quantities of immune cells that are produced by HSPCs in the bone marrow upon influenza vaccination.

Aim 2) To determine the extent to which obesity impacts bone marrow cytokine signatures and HSPC dynamics in MDS-susceptible mice. Our hypothesis is that obesity induces alterations bone marrow cytokine signatures and accelerates the timeline in which we observe disease phenotypes in a double knockout mouse model of MDS.

Chapter 2: Diet-induced Obesity Alters Hematopoiesis Dynamics Following Influenza Immunization: Bone Marrow Cytokine Signatures May Provide a Mechanism

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This work has yet to be published in a peer-reviewed journal.

Abstract

Obesity plays a significant role in public health and has been associated with poor outcomes in lasting immune protection. In addition to its contributions to influenza infection susceptibility, obesity is also known to contribute to waning immunity in influenza-immunized individuals. It has been established that compared with healthy-weight adults, the influenza vaccine is less efficacious in obese populations. We have limited understanding of the mechanisms by which this occurs. While ongoing research aims to determine immune cell mechanisms for waning immunity at sites of infection or in fully differentiated immune cells, little is known regarding how obesity impacts hematopoietic stem and progenitor cell (HSPC) dynamics upon immunization. Given that we know that immunization and obesity each have independent effects on HSPC dynamics, we hypothesized that obesity alters the type and quantity of immune cells that are produced by HSPCs following immunization. To test this hypothesis, we immunized C57/BL6 mice which were fed with low-fat (control) and high-fat (obese) for 15 weeks. Mice were subjected to caudal muscle injection with PBS (control) or flu vaccine (Flulaval quadrivalent vaccine). Our results suggest that high-fat diet flu-immunized mice altered HSPC frequency as measured by flow cytometry when compared to all other groups. Complete blood count data suggest that myeloid skewing also occurs in high-fat diet flu-immunized mice compared to low-fat diet flu-immunized mice. Further analysis of cytokines in

the bone marrow microenvironment provide some insight into how obesity may impact HSPC dynamics following immunization. Our long-term goals are to determine why obesity reduces vaccine protection against pathogens and to inform the design of future vaccine formulations or co-administered therapeutics to increase immune protection in obese individuals receiving vaccines.

Keywords: HSPCs, Cytokines, Influenza vaccine, Obesity

Introduction

Excessive deposition of body fat with a body mass index (BMI) of over 30 kg/m2 leads to obesity and disturbances in health conditions. In obesity, there is an alteration in adipokines and several other cytokines, contributing to low-grade inflammation in the adipose tissue, which leads to the development of metabolic diseases (Apovian, 2016). The effect of obesity on the immune system has poor outcomes. There are alterations in both local and systemic immunity due to metabolic stress imposed by obesity. This is due to replacement of anti-inflammatory stimulated immune cells with pro-inflammatory immune cells, which secrete pro-inflammatory cytokines (de Frel et al., 2020).

Obesity is a risk factor for many bacterial and viral infections. This is most notably the case for influenza infection. Although influenza infection typically results in relatively mild, uncomplicated symptoms in healthy populations, epidemiological data overwhelmingly suggests that obesity is a risk factor for more severe pathogenesis (Angulo-Zamudio et al., 2021), including hospitalization and death. Furthermore, in vivo studies have shown that both diet-induced and genetic mouse models of obesity are more susceptible to influenza infection and severe phenotypes associated with influenza infection. For example, Milner et al. showed that when obese mice and healthy mice are infected with influenza A virus (IAV), there is increased

inflammatory response, pulmonary edema and lung damage in the diet-induced obese and genetically ($LepRH^{-/-}$) obese mice compared with healthy weight controls (Milner et al., 2015).

Obesity also has negative effects on the immune system in influenza immunization. Following influenza immunization, more rapidly waning immunity occurs in obese populations when compared to non-obese population. This is hypothesized to be due to the alteration in innate and adaptive immune cells because of inflammation induced by pro-inflammatory cytokines. In one study (Cho et al., 2016), mice fed with high-fat diet (60% kcal fat) for 10 weeks have decreased regulatory T cells and also decreased proportion of CD-86 expressing macrophages. CD86 is essential for T-lymphocyte activation. In addition to this, influenza vaccine-induced antibodies in high-fat fed mice diminished more rapidly when compared with a control diet (Cho et al., 2016). In another study (Kim et al., 2012) relating to 2009 H1N1 pandemic influenza virus, authors have immunized (CA/07 vaccine) obese and lean mice and the humoral immunity was measured. HA specific total IgG was significantly reduced in the high-fat diet fed group when compared with a lean-diet group. Inflammatory response is also altered in vaccinated obese mice. In one study, serum levels of MCP-1 and RANTES chemokines significantly increased in obese mice compared with lean vaccinated mice. Moreover, there was increase in pro-inflammatory cytokines like TNF-α, IL-6, IL-1β on day 3 and day 8 when compared with lean immunized control mice (Kim et al., 2012).

While previous studies have focused on obesity's impact on fully differentiated cells of the immune system and cytokines measured in serum, obesity effect on HSPCs and bone marrow cytokines have not been published to our knowledge. There is a need for the studies to be conducted on HSPCs given that these cells give rise to all mature immune cells. It has been established that HSPC proliferation and differentiation has extensive effects on immune system's

ability to respond to a pathogenic insult or develop immune memory. In this study we focus on obesity impact on influenza vaccination, with respect to HSPC dynamics and bone marrow cytokine signature which influence HSPCs.

Methods

Animals: Male CD57BL/6 DIO (diet-induced obesity) and CD57BL/6 mice of 18-19 weeks were purchased from Jackson Laboratory where they were subjected to low-fat (10 kcal% fat) or high-fat (60 kcal% fat) diets for 14 weeks after weaning. Upon arrival in our facility, mice were fed and additional week while they acclimated to a new environment. Mice were fed low-fat or high-fat diets for 15 weeks total following weaning. The mice were housed in standard cages with free access to food and water and on a 12/12hr light/dark cycle.

Diet Composition: Food originated from Research Diets, Inc. The composition of each diet is outlined in the table below.

Table 2.1

Diet Macro-Nutrient Composition

Macro-nutrient composition of lard-based DIO diets (Research Diets, Inc.)				
	Control Diet D12450B (10 kcal% fat, 3.8 kcal/gram)		High Fat Diet D12492 (60 kcal% fat, 5.2 kcal/gram)	
	Gram %	**kcal %**	**Gram %**	**kcal %**
Protein	19	20	26	20
Carbohydrate	67	70	26	20
Fat	4	10	35	60
Total		100		100

Immunization: Mice were vaccinated with 50µl of the vehicle control phosphate buffered saline (PBS) or human influenza quadrivalent vaccine (Flulaval), which has four influenza strains and was diluted in PBS. We diluted the vaccine such that each mice received 0.75µg of

HA (hemagglutinin) of each flu strain within the total volume of 50µl in PBS. The site of injection was the caudal thigh muscle and route of administration was intramuscular. The mice were divided into groups such that half of the mice received a low-fat diet and half received the high-fat diet. Of the mice subjected to a low-fat diet, eight were vaccinated with vehicle control (PBS) and eight were vaccinated with the quadrivalent influenza vaccine. Of the mice subjected to a high-fat diet, eight were vaccinated with vehicle control (PBS) and eight were vaccinated with the quadrivalent influenza vaccine. A subset of mice from each group were euthanized 1 day (n=4/group) and 3 days (n=4/group) following immunization. Animals were euthanized according to our approved Institutional Animal Care and Use protocol. Samples collected were femur and tibia from both the hind limbs, blood by cardiac puncture, and spleen. All procedures were approved by the Institutional Animal Care and Use Committee of Marshall University (IACUC protocol no. 807).

Complete Blood Counts: For complete blood counts, peripheral blood was drawn into a microtainer blood collection tube coated with K2EDTA after cardiac puncture using an insulin syringe. After thoroughly mixing the microtainer tube, 100 µl of blood were transferred into a 1.5ml Eppendorf tube. This tube was used to run the blood sample in a Drew Scientific Hemavet blood analyzer.

Hematoxylin and Eosin staining: For H&E staining, we have used hematoxylin, eosin, bluing reagent, 100% ethanol and distilled water. To prepare the smears 2.5-5 µl of blood was used, so that the thin film of smear was formed by dragging the blood drop with other smear. Smear was then subjected to different stains.

Flow Cytometry: After flushing the bone marrow using 1.5ml DMEM media with 10% fetal bovine serum (FBS), the bone marrow cells were pelleted by centrifugation. The media was

removed for cytokine analysis. The bone marrow cell pellet was resuspended in PBS with 2% FBS. Red blood cells were lysed using BD PharmLyse. Cells were again centrifuged, and the cell pellet was resuspended in PBS with 2% FBS. Cells were stained with specific antibodies attached to a fluorophore (Table 2.2). The stained cells were incubated for 1 hour in dark at 4˚C. They were then centrifuged at 500 rcf to retain the stained cells and remove the PBS. The samples were run on a NovoCyte 2000, and results were analyzed using FlowJo software.

Table 2.2

Antibodies Used for Flow Cytometry

Antibodies used for HSPC staining		
Antibody	**Fluorophore**	**Catalog Number**
Lineage	APC	51-9003632
Ly-6A/E	BB515	565397
CD117	PE	565397

Note. Antibodies were purchased from BD Biosciences (catalogue no: 51-9003632, 565397, 565397). Lineage cocktail components include clone 145-2C11, which recognizes Mouse CD3e; M1/70, which recognizes CD11b; RA3-6B2, which recognizes CD45R/B220; TER-119, which recognizes Ly-76, mouse erythroid cells; and RB6-8C5, which recognizes Ly-6G and Ly-6C.

Cytokine Analysis: Media used to flush the bone marrow was isolated following centrifugation of cells. The media was subjected to cytokine analysis using C3 cytokine membranes (Raybiotech). The C3 membranes have a unique set of cytokines (Appendix C). These cytokine arrays work on the principle of sandwich ELISA. Results were detected using a chemiluminescence imager, Densitometry was performed using ImageJ software.

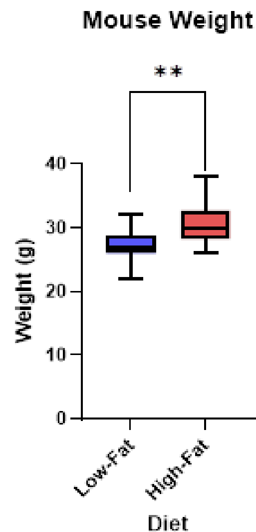
Results

High-Fat Diet Induces Increased Weight in C57BL/6 Mice

Mice were weighed to ensure that the high-fat diet induced obesity in the mice we obtained from The Jackon Laboratory. Mice fed a high-fat diet were significantly heavier (3.438 ± 0.9616g; p=0.0012) than mice fed a low-fat diet (Figure 2.1). There was no significant change in weight when comparing mice euthanized one day vs. three days following influenza immunization, suggesting that immunization did not affect overall weight of the mice (data not shown).

Figure 2.1

High-Fat Diet Induces Weight Gain in C57BL/6 Mice



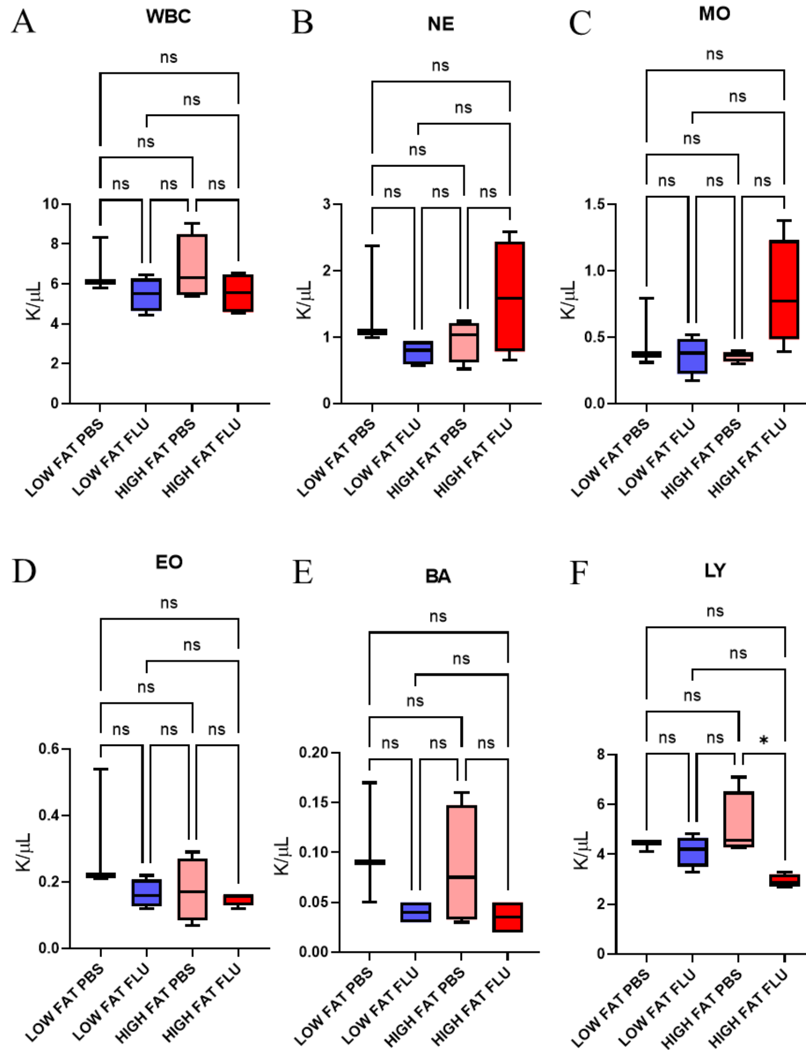
Note. Male mice fed a low-fat control or high-fat diet for 15 weeks following weaning at 4 weeks of age were weighed. Results were analyzed using unpaired t-tests. Two asterisks (**) indicate $p < 0.01$; N=8 mice/group.

Diet Induced Obesity Alters Complete Blood Counts Following Influenza Immunization in C57BL/6 Mice

To test our hypothesis that obesity contributes to alterations in hematopoiesis following influenza immunization, we performed complete blood counts on peripheral blood of mice collected three days following immunization. While there were no significant differences in total white blood cell counts across groups (Figure 2.2), differential analysis showed trends of increased myeloid cell types in neutrophils ($p=0.21$) and monocytes ($p=0.07$) (Figure 2.2B and 2.2C, respectively) present in peripheral blood of influenza-immunized high-fat diet mice when compared to all other groups. Other myeloid cell types, eosinophils and basophils, were not significantly different or trending any way in influenza-immunized high-fat diet mice when compared to other groups (Figure 2.2D and 2.2E, respectively). In analysis of lymphocytes, influenza-immunized high-fat diet mice exhibited significantly lower total lymphocytes compared to high-fat diet PBS-injected mice ($p= 0.0114$) and exhibited a trend of lower total lymphocytes when compared to all other groups (Figure 2.2).

Figure 2.1

High-Fat Diet Induces Alterations in Differential White Blood Cell Counts in Peripheral Blood of Flu-Immunized Mice

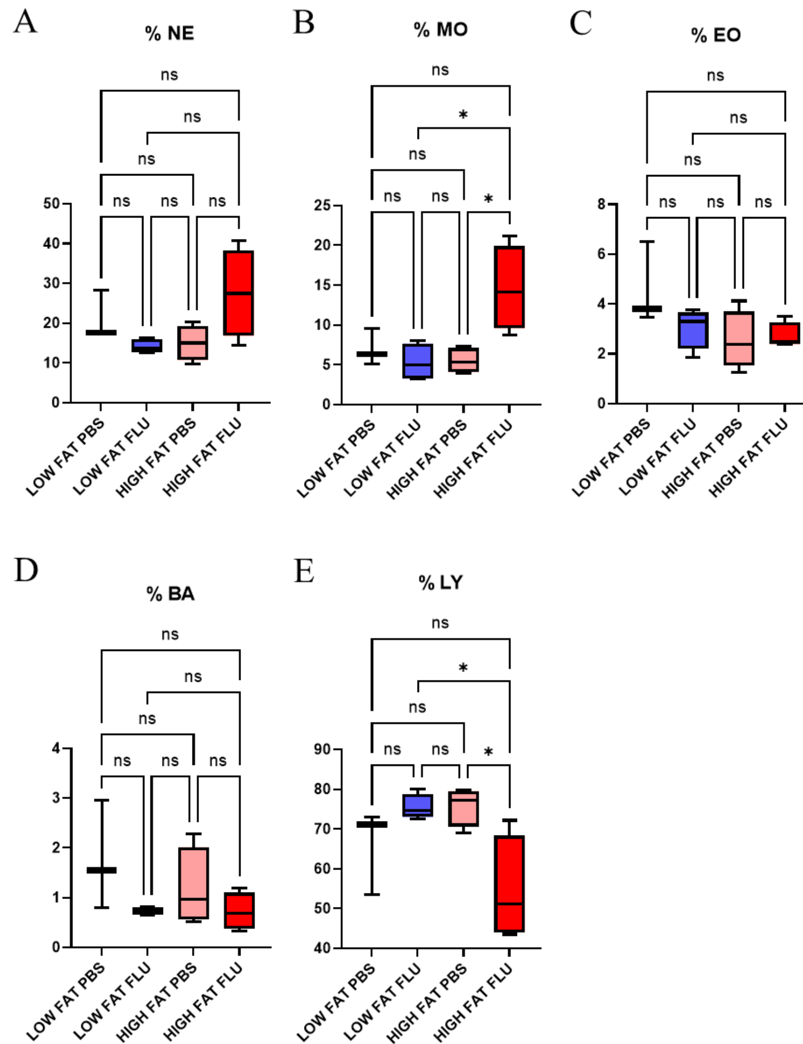


Note. C57BL/6 male mice fed a low-fat or high-fat diet for 15 weeks were injected with PBS or influenza quadrivalent vaccine (Flulaval), Three days following immunization peripheral blood was collected and analyzed on a Drew Scientific Hemavet blood analyzer for total counts of white blood cells (A), neutrophils (B), monocytes (C), eosinophils (D), basophils (E), and lymphocytes (F). One asterisk (*) indicate $p < 0.05$; N=3-4 mice/group.

Given that proportions of cell types within the blood is also relevant to hematopoiesis and immune response, differential white blood cells were also analyzed for the proportion of blood they compose. Regarding myeloid cells, high-fat diet flu-immunized mice showed trends of increased neutrophil proportions (p=0.07) in the peripheral blood when compared to all other groups (Figure 2.3A). Proportions of monocytes in the peripheral blood were significantly higher in high-fat diet flu-immunized mice when compared to low-fat diet flu-immunized mice (p=0.0107) and high-fat diet PBS-injected mice (p= 0.0124) (Figure 2.3B). There were no significant differences or notable trends regarding eosinophil and basophil proportions (Figure 2.3C and 2.3D respectively). Regarding lymphocytes proportions, high-fat diet flu-immunized mice showed significantly lower proportions of lymphocytes in peripheral blood when compared to low-fat diet flu-immunized mice (p= 0.0275) and high-fat diet PBS-injected mice (p= 0.0252).

Figure 2.3

High-Fat Diet Induces Alterations in Differential White Blood Cell Proportions in Peripheral Blood of Flu-Immunized Mice



Note. C57BL/6 male mice fed a low-fat or high-fat diet for 15 weeks were injected with PBS or influenza quadrivalent vaccine (Flulaval), Three days following immunization peripheral blood was collected and analyzed on a Drew Scientific Hemavet blood analyzer for proportions of white blood cells (A), neutrophils (B), monocytes (C), eosinophils (D), basophils (E), and lymphocytes (F) in the peripheral blood. One asterisk (*) indicate $p < 0.05$; N=3-4 mice/group.

Analysis of the following complete blood count parameters showed no significant differences or notable trends across groups (data not shown): red blood cells, hemoglobin, hematocrit, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelets, and mean platelet volume.

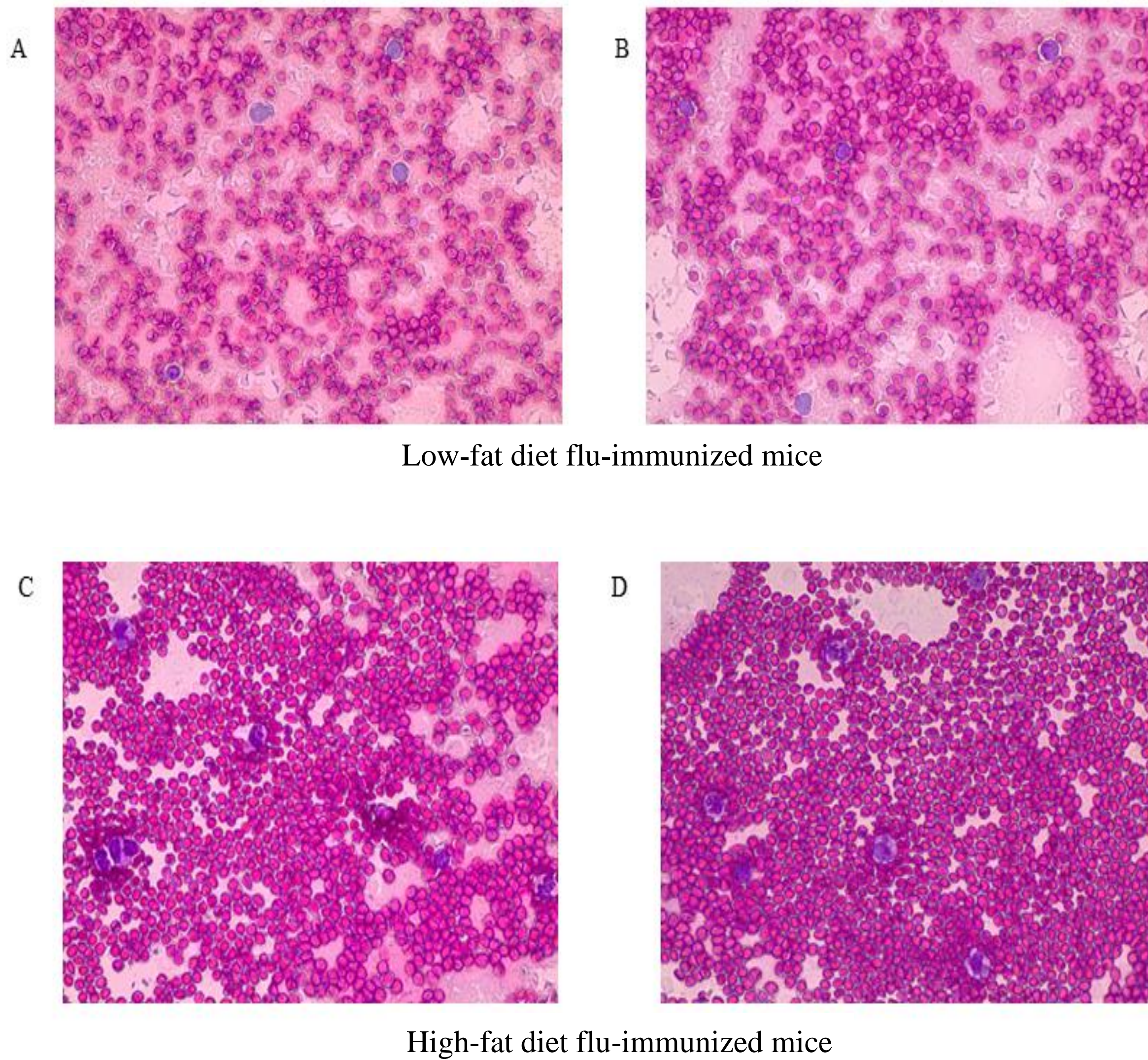
Taken together, trends of increased myeloid cells and cell proportions and trends of decreased lymphocytes and lymphocyte cell proportions in the high-fat diet flu-immunized mice when compared to other groups indicates that obesity may influence myeloid skewing in hematopoiesis. This data is supported with the blood smears shown below.

Diet Induced Obesity Alters Specialized Immune Cells Following Influenza Immunization When Observed Under Blood Smears.

Blood smears from low-fat diet flu-immunized mice exhibit more lymphocytes (Figure 2.4A, 2.4B) when compared to high-fat diet flu-immunized (Figure 2.4C, 2.4D). High-fat diet flu-immunized mice exhibit higher frequencies of neutrophils and reactive monocytes (Figure 2.4C, 2.4D)**.** when compared to low-fat diet flu-immunized mice ((Figure 2.4A, 2.4B)**.**

Figure 2.4

High-Fat Diet Induces Alterations in Specialized Immune Cells in Peripheral Blood of Flu-Immunized Mice



Low-fat diet flu-immunized mice

High-fat diet flu-immunized mice

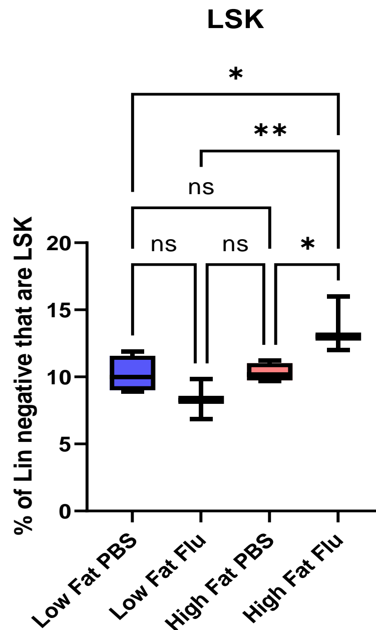
Note. C57BL/6 male mice fed a low-fat or high-fat diet for 15 weeks were injected with PBS or influenza quadrivalent vaccine (Flulaval), Three days following immunization peripheral blood was collected and staining was performed using H & E staining protocol and observed under the magnification of 60x.

Diet Induced Obesity Alters Hematopoietic Stem and Progenitor Cell Frequency Following Influenza Immunization in C57BL/6 Mice

To further understand the source of obesity-induced alterations in myeloid and lymphoid cell counts and proportions in the peripheral blood, we assessed the bone marrow for frequency of HSPCs. While it is known that obesity promotes myelopoiesis over lymphopoiesis, this alone does not explain the how peripheral blood cell counts are altered between high-fat diet PBS-injected mice and high-fat diet flu-immunized mice. Given that it is known that immunization (or pathogen exposure) contributes to hematopoietic stem cell expansion, we hypothesized that high-fat diet flu-immunized mice may also exhibit some degree of bone marrow HSPC expansion. Bone marrow was extracted from mice three days following PBS-injection or flu-immunization. Cells were stained and analyzed by flow cytometry using standard methods for evaluating HSPCs, which are lineage negative, Sca-1 positive, and c-kit positive (LSK) cells. Results indicate that of the lineage negative cells of the bone marrow, LSK cells are significantly increase in high-fat diet flu-immunized mice when compared to all other groups (Figure 2.5).

Figure 2.5

High-Fat Diet Induces Hematopoietic Stem and Progenitor cell expansion in the bone morrow of flu-immunized mice.



Note. C57BL/6 male mice fed a low-fat or high-fat diet for 15 weeks were injected with PBS or influenza quadrivalent vaccine (Flulaval), Three days following immunization, bone marrow was extracted and stained for lineage panel, Sca-1, and c-kit. Cells were analyzed by flow cytometry using a NovoCyte 2000. Using FlowJo software, Lineage negative, Sca-1 positive, c-kit positive (LSK) cells were determined for each group. One asterisk (*) indicate $p < 0.05$; Two asterisks (**) indicate $p < 0.01$; N=3-4 mice/group.

Obesity Influences Cytokine Signatures in the Bone Marrow of Flu-Immunized Mice

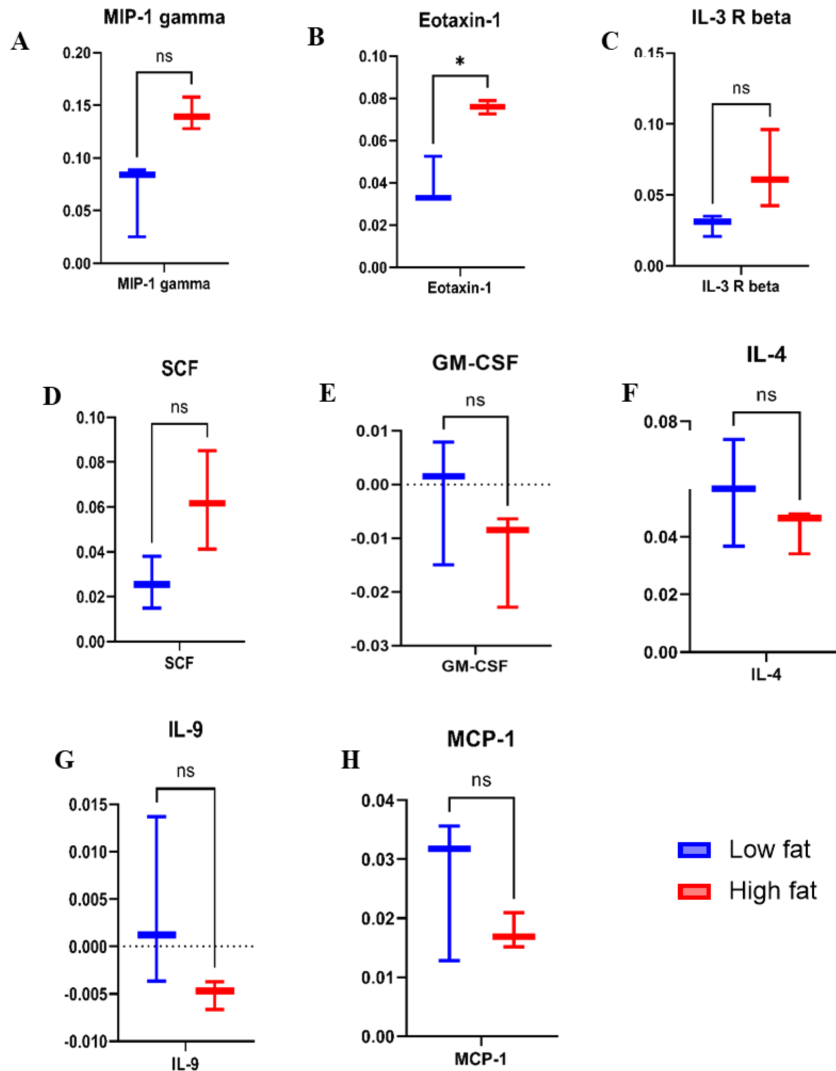
Given our understanding of the role of obesity in influencing cytokines available in the bone marrow microenvironment, we hypothesized that obesity may elicit its effects on HSPC expansion and peripheral blood changes following flu-immunization by altering the unique

cytokine signatures present in the bone marrow. At a time point of three days following flu-immunization of mice fed a low-fat or high-fat diet for 15 weeks, we extracted bone marrow. We isolated the media flushed through the bones from the cells of the bone marrow. Media was then analyzed for cytokines using RayBiotech C3 cytokine arrays.

Cytokines MIP-1 gamma, Eotaxin-1, MCP-1, IL-3R beta, IL-4, SCF, GM-CSF, MCP-1 showed particular trends of alteration between low-fat and high-fat diet flu-immunized mice (Figure 2.6 A-H). Eotaxin-1, however, was the only significantly altered cytokine as analyzed by unpaired t-tests (Figure 2.6B). While statistical significance is powerful, biological significance may still exist in these cytokine trends, especially when the information is collectively considered.

Figure 2.6

Obesity Contributes to Alterations in Bone Marrow Cytokine Signatures Following Flu-Immunization in Mice



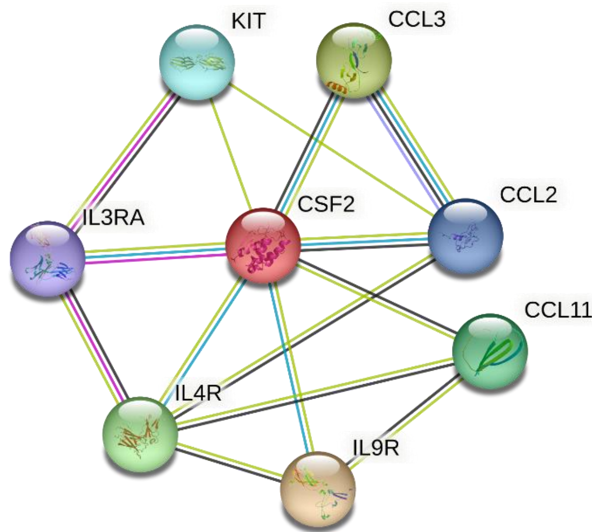
Note. C57BL/6 male mice fed a low-fat or high-fat diet for 15 weeks were immunized with an influenza quadrivalent vaccine (Flulaval). Three days following immunization, bone marrow was extracted using a method by which media is flushed through the femurs and tibias. Bone marrow was then subjected to centrifugation. Media was isolated and analyzed following manufacturer's instructions for C3 cytokine arrays. Cytokines most notably showing trends or significant

differences are displayed as follows: MIP-1gamma (A), Eotaxin (B), IL-3R beta (C), SCF (D), GM-CSF (E), IL-4 (F), IL-9 (G), and MCP-1 (H). One asterisk (*) indicate $p < 0.05$; N=3 mice/group.

When the trending cytokines are listed in the String database, they are shown to interact with one another (Figure 2.7).

Figure 2.7

Cytokines That Show Differential Trends in the Bone Marrow of High-Fat Diet Flu-Immunized Mice and Low-Fat Diet Flu-Immunized Mice Interact.



Note. MIP-1gamma (CCL9), Eotaxin-1 (CCL11), IL-3R beta, SCF(KIT), GM-CSF (CSF2), IL-4, IL-9, and MCP-1(CCL2) were entered in String database. When a cytokine was not available in the database, its receptor or closest homolog was selected. Line color indicates the type of interaction evidence and line thickness indicates the strength of data support. Curated database interactions (turquoise blue), experimentally determined (magenta), text-mining (yellow), co-expression (black), protein homology (light purple).

Discussion

In this study, we aimed to better understand how obesity influences HSPC dynamics following influenza immunization. Results indicated that obesity contributes to vaccine-induced alterations in peripheral blood and HSPC frequency within the bone marrow. Upon investigating the cytokines in the bone marrow to better understand the mechanisms underlying these observations, we discovered trends that may provide insight into changes in the bone marrow microenvironment that contribute to decreased influenza vaccine efficacy in obese individuals. When considered individually, only one cytokine, eotaxin-1, showed significant change in vaccination of diet-induced obese mice when compared to non-obese mice. However, when taking into account how other cytokines with differing trends between the two groups interact, it provides a better understanding of the bone marrow microenvironment changes that occur when obesity is factor in immunization. These cytokines are further discussed below.

MIP-1 gamma, also known as CCL9, belongs to the CC chemokine family. It plays an important role in immune cell trafficking and activation. It binds to CCR1 and CCR5 receptors expressed on immune cells including HSPCs. It contains functional binding sites for early B cell factor and plays crucial role in regulating B lymphopoiesis (Mohamadzadeh et al., 1996) (Lagergren et al., 2007). Furthermore, it has been shown to increase NF-kappa B activation. NF-kappa B plays an important role in inflammation associated with LSK expansion. While not significant, it shows a trend of increase in high-fat diet flu-immunized mice compared with low-fat diet flu-immunized mice (Figure 2.6A).

Eotaxin-1, also known as CCL11, is a CC chemokine that plays an important role in attracting immune cells including eosinophils, basophils, and Th2 lymphocytes to the site of infection. Eotaxin is also known to promote the generation of mast cells and myeloid progenitor

cells. It performs this task in synergistic combination with SCF. Furthermore, eotaxin promotes angiogenesis and inflammation. Regarding influenza immunization, eotaxin levels are altered after immunization with influenza vaccine in patients who have allergic bronchial asthma. (Jahnz-Rozyk et al., 2004; Radinger et al., 2004) Eotaxin is significantly upregulated in flu-immunized mice that consume a high-fat compared to low-fat diet (Figure 2.6B). This trend may help to explain why myeloid cells are increased in high-fat diet flu-immunized mice.

IL-3R beta is a subunit of the receptor for IL-3, a cytokine that is involved in the regulation of hematopoiesis. When the beta subunit binds with IL-3, it triggers a series of intracellular signaling events leads to the activation of various transcription factors and production of proteins that regulate growth and differentiation (Hara & Miyajima, 1996). It has synergistic functions with GM-CSF and IL-5 when they act on the same cells. IL-3 is necessary for sending signals for DNA synthesis, which in turn, leads to HSPC proliferation. This cytokine has trends of increased availability in the bone marrow of high-fat diet flu-immunized mice when compared to low-fat diet flu-immunized mice (Figure 2.6C). Its impact on HSPC proliferation may explain to some degree why HSPC frequency is increased in high-fat diet flu-immunized mice.

Stem Cell Factor (SCF), also known as c-kit ligand, plays a crucial role in hematopoiesis including the proliferation and maintenance of hematopoietic stem and progenitor cells. SCF is produced by bone marrow where HSPCs reside and binds to the receptor c-kit on the surface of HSPCs. Bone marrow adipose tissue interacts with local HSPCs and bone cells and contributes to metabolism through secretion of SCF, leptin, and adiponectin (Li & MacDougald, 2019). SCF shows trends of increased presence in the bone marrow of high-fat diet flu-immunized mice compared with low-fat diet flu-immunized mice. (Figure 2.6D). This is not surprising, given its

role in maintaining stemness of HSPCs. This may also help explain to some degree why HSPC frequency is increased in high-fat diet flu-immunized mice.

GM-CSF promotes the survival and maturation of HSPCs into mature granulocytes and macrophages (Hercus et al., 2009). Surprisingly, it shows decreasing trends in high-fat diet flu-immunized mice compared with low-fat diet immunized mice (Figure 2.6E). This may be explained in part by its reliance on IL-4.

IL-4 is a cytokine that plays an important role in antibody production. It also regulates hematopoiesis and HSPCs by directly acting on HSPCs through receptor IL-4R. It is involved in differentiation of HSPCs into various lineages like T cells, B cells, and myeloid cells. IL-4 stimulates the production of stromal cells like fibroblasts and osteoblasts, which are important for maintenance and regulation of HSPCs.(Sonoda, 1994) (Keller et al., 1994). IL-4 inhibits IL-3-dependent colony formation. Macrophage colony formation supported by IL-3 and M-CSF, GM-CSF, or M-CSF alone is inhibited by IL-4. IL-4 enhances the release of GM-CSF or G-CSF from immune cells. IL-4 shows decreasing trends in high-fat diet flu-immunized mice when compared with low-fat diet flu-immunized mice (Figure 2.6F). Given its function in enhancing GM-CSF, this may be one reason GM-CSF is also slightly decreased in high-fat diet flu-immunized mice. Given its roles in lymphocyte development, IL-4 decrease may explain to some extent why lymphocytes are significantly reduced in the peripheral blood of high-fat diet flu-immunized mice.

IL-9 is a cytokine produced mainly by T lymphocytes. Regarding stem cells, it has been shown to slow the development of mast cells from CD34+ progenitors along with SCF (Matsuzawa et al., 2003). More recently, Il-9 has been found to serve a role in regulating inflammatory immunity. It has shown trends of decreasing in high-fat diet flu-immunized mice

when compared with low-fat diet flu-immunized mice (Figure 2.6G). Interestingly, IL-9 has been shown to activate production of exotoxin, suggesting that the high exotoxin in high-fat diet flu-immunized mice may create negative feedback, thus suppressing IL-9 production in the bone marrow microenvironment.

MCP-1 (monocyte chemoattractant protein) also known as CCL-2, plays a role in regulation of hematopoiesis by controlling the migration and recruitment of monocytes and other immune cells to the site of inflammation.(Xu et al., 1999). MCP-1 is known to elicit myelosuppressive effects. MCP-1 shows trends of decreased presence in the bone marrow of high-fat diet flu-immunized mice compared with low-fat diet flu-immunized mice. (Figure 2.6H). Decreases in this myelosuppressive cytokine may help to explain why myeloid cell proportions are increased in high-fat diet flu-immunized mice.

Taken together, our data provides novel insight on how obesity may lower vaccine efficacy in obese populations due to its impact on the bone marrow microenvironment and HSPC dynamics, which ultimately determine the types and quantities of cells mobilized from the bone marrow to the blood and tissues. Alternate interpretations for blood counts apart from alterations in hematopoiesis include the potential of lymphocyte mobilization to lymph nodes for expansion following immunization. Another possibility may include cell death of lymphocytes due to the effect of antigens (Vasseur et al., 1999), (Wahl et al., 1993). Future experiments will involve stimulation or inhibiting identified cytokines during flu immunization in obese mice in attempts to rescue phenotypes associated with low-fat diet flu-immunized mice. Future experiments may also involve studies performed at additional time points following immunization. One caveat to our work is that our studies include only male mice. Male mice were studied because female C57BL/6 mice are resistant to diet-induced obesity. Day 3 was chosen to euthanize mice after

immunization, as it elicits major immune response on day3 compared to day 1, as HSPCs are early cells it doesn't require much time to get responded to an antigen (Varney et al., 2018). Future studies may also include female mice to better understand dietary impact regardless of weight gain. Other limitations include the sample size for each group, which may be deviating our results from reaching statistical significance. Future studies also include experiments with increased sample size. In closing, we anticipate that our work will contribute to future formulations of flu-vaccine or co-administered therapeutics at the time of immunization in order to increase long-term immune protection in obese individuals receiving the flu vaccine. Moreover, these studies may provide information that may globally impact future immunization strategies in for other vaccines that may show waning immunity in obese populations

Chapter 3: Western Diet Contributes to Sex-Specific Alterations in Bone Marrow Cytokines in MDS-Susceptible Mice

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Abstract

Recent studies suggest that obesity due to Western diet contributes to hematologic malignancies. Previously, we have observed that Western diet contributes to more rapid initiation and progression of cancer phenotypes in an established mouse model of myelodysplastic syndromes (MDS). We hypothesized that this acceleration of initiation and progression of MDS was due in part to the presence of unique cytokine signatures in the bone marrow microenvironment of mice fed the Western diet compared to those on a low-fat control diet. Del5q MDS is associated with loss of expression of immune genes on human chromosome 5q. Combined deletion of two of the genes, TIFAB and miR-146a, in mice recapitulates del5q MDS disease phenotypes. In this study, Tifab-/-;miR146a-/- double knock out (DKO) mice placed on a Western diet or low-fat control diet for 15 weeks. Mice on the Western diet exhibit significant weight gain confirming that DKO mice are susceptible to diet-induced obesity. After 15 weeks of their assigned diets, bone marrow was extracted by a method of flushing media through the femurs and tibias. Media was then isolated from the bone marrow cells by centrifugation. Media was then subjected to cytokine array analysis. Western diet induced unique cytokine signatures in a sex-specific manner. Male mice fed a Western diet showed significantly increased IL-11, significantly decreased HGF, and trends of decreased Galectin-1 compared with other groups.

These findings underscore the potential importance of bone marrow cytokine signature alterations in diet-induced onset and progression of MDS/AML.

Introduction

MDS and Chronic Innate Immune Signaling

Myelodysplastic syndromes (MDS) refer to a group of hematopoietic stem and progenitor cell (HSPC) disorders clinically associated with myeloid dysplasia, ineffective hematopoiesis, genomic instability, and increased risk for cancer progression to acute myeloid leukemia (AML). Despite recent progress, currently the only method to cure MDS is a hematopoietic stem cell transplant. Many patients with MDS are not eligible for this, and transplantation poses many risks of complications, some that are potentially fatal. A fairly new area for drug targets in MDS and AML includes mediators of innate immune signaling. Pro-inflammatory influences on MDS have been reported for many years. Recent findings, however, provide evidence that support direct roles of chronic innate immune signaling and downstream inflammatory pathways in MDS and AML pathogenesis (Barreyro et al., 2018; Varney, Melgar, et al., 2015). Moreover, dysregulation of innate immune signaling genes is common in MDS HSPCs (Starczynowski, 2014). Innate immune signaling in HSPCs promotes selective pressures on these primitive cells, which in turn contributes to trained immunity and clonal hematopoiesis of myeloid-biased HSPCs (Mitroulis et al., 2018). It is hypothesized that trained innate immune memory, which refers to long-term changes in the reactivity of innate immune cells exposed to stimuli, precedes MDS and that this could be driven by continuous innate immune signaling (Kaufmann et al., 2018). This study will investigate Western diet as a contributor to initiation and progression of MDS and AML. It is well established that Western diet leads to chronic innate immune signaling and inflammation (Monteiro & Azevedo, 2010).

Genetics of MDS and MDS-Susceptible Mouse Models

Genetic deletion is a driver of chronic innate immune signaling in MDS. Loss of gene expression from chromosome (chr) 5q results in increased innate immune signaling and pro-inflammatory responses in HSPCs as well as transformation to MDS and AML (Varney, Melgar, et al., 2015). Interstitial deletion of chromosome 5q is the most frequent cytogenetic alteration in MDS. This deletion results in reduced expression of numerous genes, and the extent of the deletion determines disease severity (Jerez et al., 2012). Two commonly deleted regions mapped to chromosome chr 5q are located at chr 5q31.1 and chr 5q33.3 and each span ~1 Mb (Zhao et al., 1997). Loss of several genes within and between these regions have been shown to contribute to MDS (Varney, Niederkorn, et al., 2015) (Boultwood et al., 2007). We have identified that two of these genes, TRAF-interacting protein with forkhead-associated domain B (TIFAB) and microRNA-146a (miR-146a), function to regulate innate immune signaling in HSPCs. In this study, we use an established mouse model of MDS. Tifab-/-;miR146a-/- double knock out (DKO) mice recapitulate disease phenotypes associated with long-spanning deletions of chr5q. These mice display decreased survival, increased dysplasia, increased HSPC defects, and increased toll-like receptor 4 (TLR4) signaling when compared to Tifab-/- or miR146a-/-single knock out models of disease. (Varney, Melgar, et al., 2015; Varney, Niederkorn, et al., 2015).

Obesity as a Driver of Chronic Innate Immune Signaling in MDS

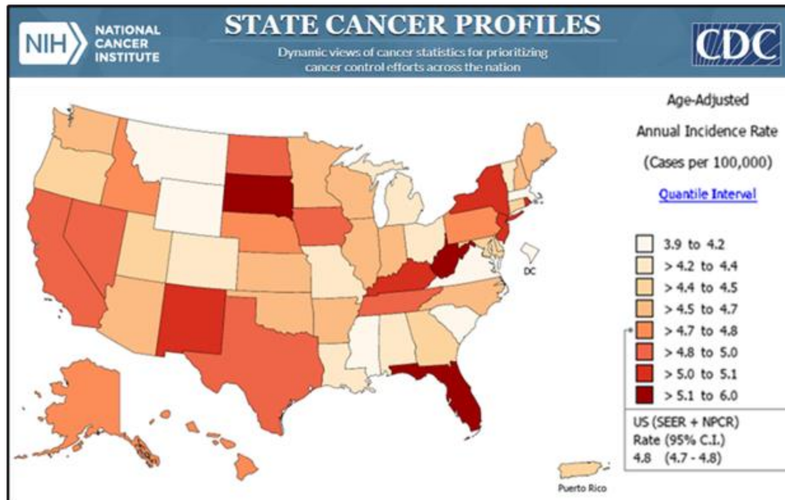
An under-investigated area of MDS and AML is the contribution of obesity to inflammation in HSPCs. Obesity is associated with increased infection susceptibility, lower vaccine efficacy, and increased chronic inflammation (Almond et al., 2013) (Cho et al., 2016) (Monteiro & Azevedo, 2010). Inflammation elicits immense changes in HSPCs that lead to skewed differentiation and increased probability of HSPC malignant transformation (Barreyro et

al., 2018). In the last decade, obesity has been associated with most forms of tumor-based cancer, but has also been linked to hematological malignancies (Calle et al., 2003). Obese patients have increased incidence of MDS and leukemia (Ma et al., 2009) (Murphy et al., 2013). Furthermore, excess fat mass is linked to higher incidence of childhood leukemia and lower overall survival of pediatric leukemia patients (Orgel et al., 2016). According to "The State of Obesity: Better Policies for a Healthier America," West Virginia has the highest adult obesity rate and second highest youth obesity rate in the nation. Given this incidence of obesity and that West Virginia also has the highest incidence of leukemia under the age of 50 in the U.S. (Figure 3.1), understanding mechanisms by which obesity drives HSPC transformation, MDS, and AML is highly relevant to our local as well as and global health.

Given that stem cell transplantation is a costly cure with limits to eligibility and risky complications, there is a critical need to identify novel therapeutic opportunities for MDS and AML patients as well as preventatives for patients with known susceptibility/risk for the diseases. Understanding the mechanisms by which diet-induced obesity drives chronic innate immune signaling and inflammation and subsequent HSPC transformation will inform the design of future therapeutics/immunotherapies for these hematologic diseases.

Figure 3.1

Incidence of Leukemia in the U.S. by State for Patients <50 Years Old



Note. This map was generated using National Institute of Health's interactive maps for state cancer profiles feature, selecting Leukemia incidence. https://statecancerprofiles.cancer.gov

Methods

Animals: Tifab-/-;miR-146a-/- (DKO) mice were provided by the lab of Dr. Daniel Starczynowski at Cincinnati Children's Hospital Medical Center. Mice were fed high-fat (40 kcal% fat) or low-fat diets (10 kcal % fat), respectively, for 15 weeks following weaning, as described in chapter 2. The mice were housed in standard cages with free access to food and water and on a 12/12hr light/dark cycle. After 15 weeks on the diet, animals were euthanized according to our approved Institutional Animal Care and Use protocol. Bone marrow was extracted from two femurs and one tibia from hind limbs of the mice. All procedures were approved by the Institutional Animal Care and Use Committee of Marshall University (IACUC protocol no. 726).

Diet Composition: Food originated from Research Diets, Inc. The composition of each diet is outlined in the table below. It should be noted that the Western (high-fat) diet also includes 1.5g cholesterol.

Table 3.1

Diet Macro-Nutrient Composition

Macro-nutrient composition of lard-based DIO diets (Research Diets, Inc.)				
	Control Diet 98121701 (10 kcal% fat, 3.91 kcal/gram)		Western Diet D12079B (40 kcal% fat, 4.68 kcal/gram)	
	Gram %	**kcal %**	**Gram %**	**kcal %**
Protein	17	17	20	17
Carbohydrate	71	73	50	43
Fat	4	10	21	40
Total		100		100

Cytokine Analysis: DMEM with 10% FBS in a volume of 1.5 ml was flushed through the bones. Media used to flush the bone marrow was then isolated following centrifugation to pellet bone marrow cells. The media was subjected to cytokine analysis using C3, C4, and C5 cytokine membranes (Raybiotech). These membranes each have a unique set of cytokines. These cytokine arrays work on the principle of sandwich ELISA. Results were detected using a chemiluminescence imager. Densitometry was performed using ImageJ software.

Results

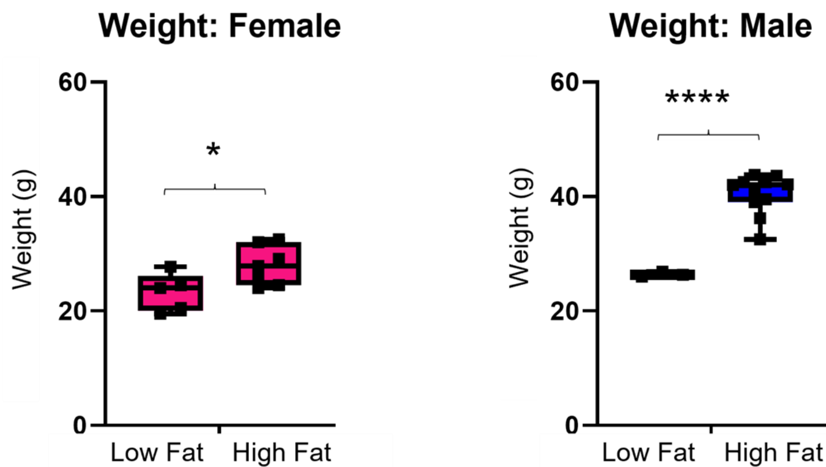
Western Diet Induces More Significant Weight Gain in Male DKO Mice

To understand how obesity contributes to initiation and progression of MDS/AML, we first validated that the Western diet induces obesity in DKO mice. Male and female mice were placed on a low-fat control or high-fat Western diet for 15 weeks. Mice were then weighed. Male mice fed a Western diet weight 14.03 ± 2.057g more than those fed a low-fat diet ($p = <0.0001$)

(Figure 3.2). At week 0 male mice weigh 10.8g .There was no significant difference in weight at week 0 between male and female with normal diets. Female mice fed a Western diet weight 4.746 ± 1.974g more than those fed a low-fat diet (p=0.0371). At week 0 female mice weigh 11.2g.

Figure3.2

Western Diet Induces More Significant Weight Gain in Male DKO Mice



Note. Male and female mice were placed on a low-fat control or high-fat Western diet for 15 weeks. Mice were then weighed. (*) indicate $p < 0.05$; Four asterisks (****) indicate $p < 0.0001$; N=5-11 mice/group.

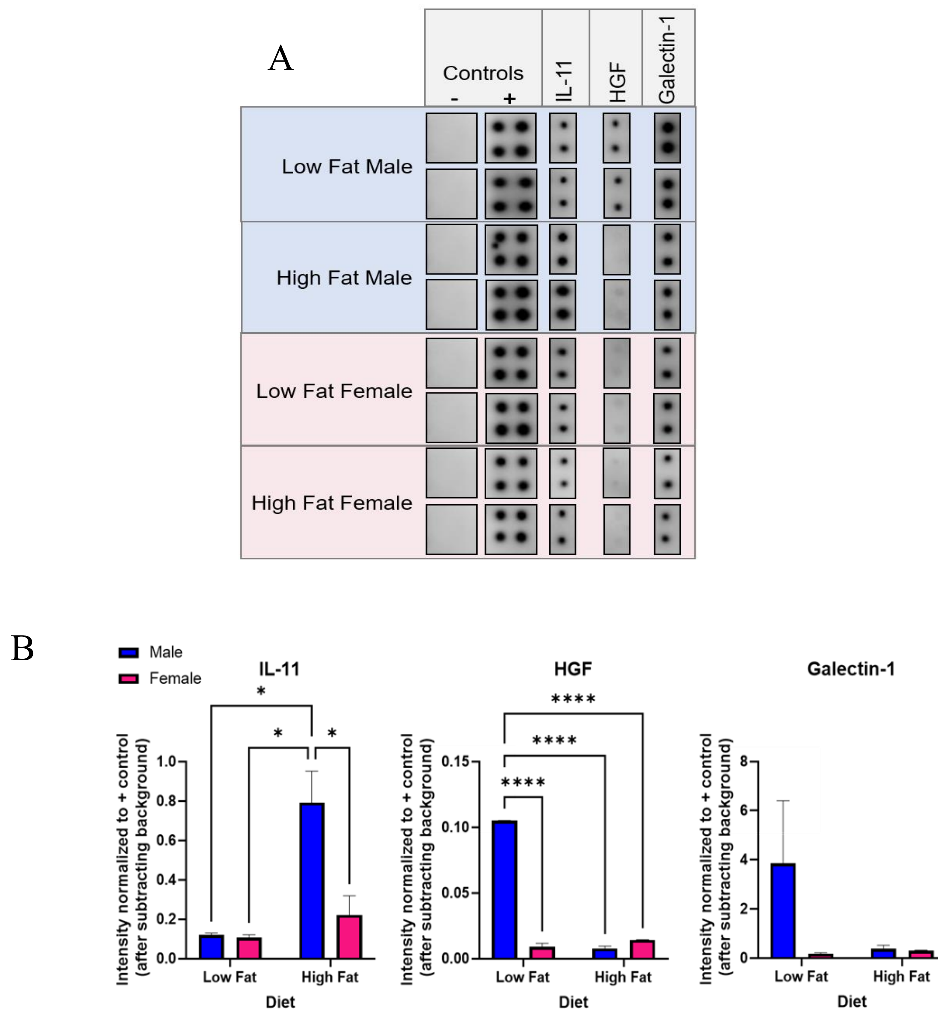
Western Diet Induces Sex-Specific Alterations in Bone Marrow Cytokines

Given that Western diet induced significant weight gain in our MDS-susceptible DKO mouse model, we tested our hypothesis that Western diet contributes to alterations in cytokines in the bone marrow microenvironment of DKO mice. After 15 weeks on their respective diets, DKO mice were euthanized. Bone marrow was extracted using the method of flushing media through the bones. Media was then isolated from bone marrow cells by centrifugation and

subjected to RayBiotech C3, C4, and C5 cytokine arrays, in which there are 144 different cytokines (Appendix C). Of these arrays, differential cytokine expression was detected on the C5 arrays. Differential expression was noticeable for cytokines IL-11, HGF, and Galectin-1 in male high-fat diet mice. Images of these arrays are shown in Figure 3.3A. Calculation of relative cytokine intensity is shown in Figure 3.3B.

Figure 3.3

Western Diet Induces Differential Cytokine Expression of Bone Marrow Cytokines IL-11, HGF, and Gelectin-1



Note. Male and female mice were placed on a low-fat control or high-fat Western diet for 15 weeks. Bone marrow was extracted using a method of flushing media through the bones. Media was then isolated from bone marrow by centrifugation to pellet bone marrow cells. Bone marrow media was then subjected to RayBiotech C3, C4, and C5 cytokine array analysis using manufacturer's instructions. Images of select cytokines on the C5 array are shown (A). Analysis of the cytokine intensity was measured using Image J software. Cytokine intensity was normalized to the array positive control following the subtraction of background (negative control). (*) indicate $p < 0.05$; Four asterisks (****) indicate $p < 0.0001$; N=5-11 mice/group.

Discussion

Western diet-induced obesity appears to have sex-specific consequences in DKO mice. While female DKO mice are susceptible to weight gain after 15 weeks on a Western diet, they appear to be less susceptible to diet-induced obesity than male DKO mice. It has been shown that female C57/BL6 mice have resistance to diet-induced obesity (Siffert, 1987). Our DKO model was generated using a C57/BL6 background strain. Future work in determining the impact of sex on disease outcomes in DKO mice fed a Western diet is warranted. With normal diets immediately after weaning there was no significant sex-specific differences. The weight gain in DKO mice model is more than the weight gain seen in C57BL/6 mice in chapter 2. This difference may be due to the DKO mice susceptibility to obesity or it may be due to the fact that the high-fat diet in the studies in chapter 3 contained 1.5 g of cholesterol. There was no added cholesterol in the studies in the high-fat diet in Chapter 2.

Of the cytokines that show significance or trends of differential availability in the bone marrow male DKO mice fed a Western diet, each are highly involved in HSPC frequency and/or function. IL-11 is established to supports the proliferation of HSCs and megakaryocyte

progenitor cells (Du & Williams, 1994) (Turner et al., 1996). MDS/AML originated from proliferation of dysfunctional HSPCs (Xing et al., 2022). Perhaps obesity-induced availability of IL-11 in the bone marrow microenvironment aids in this process and therefore contributes to acceleration of initiation and progression of MDS/AML. Furthermore, dysmegakaryopoiesis is prevalent in MDS (Falconi et al., 2019). Disproportionate increases in megakaryocyte precursors and changes in morphology of megakaryocytes in MDS are established as a consequence of defects in hematopoiesis. The role of IL-11 as a driver of inflammation has also recently become a highly researched topic in the field of oncology (Xu et al., 2016).

Regarding HGF, this cytokine is known to promote survival and differentiation of HSPCs. It mobilizes bone marrow HSPCs to the peripheral blood. HGF is also know to support the differentiation of progenitors in megakaryocyte lineage (Ikehara, 1996). Decreases in the availability of this cytokine in the bone marrow of male mice may complement the increase in IL-11. Taken together, this indicates that in the bone marrow microenvironment, HSPCs are proliferating, failing to differentiate and mobilize, and causing dysmegakaryopoiesis by failing to allow the differentiation of megakaryocytes and simultaneously inducing the proliferation of megakaryocyte progenitors.

Though Galectin-1 decrease in male high-fat diet DKO mice are not statistically significant, this trend may have a biologically significant role. Galectin-1 mediates interactions between hematopoietic cells and the stromal microenvironment and acts as a classical proapoptotic factor for the premature hematopoietic cells (Vas et al., 2005) (Rabinovich & Vidal, 2011). Decrease in galactin-1 in the microenvironment of make DKO mice fed a high-fat diet may disrupt the microenvironment cell-to-cell communication and prevent defective HSPCs from undergoing apoptosis.

Taken together, our observations indicate that diet-induced obesity may contribute to accelerated initiation of MDS or progression of the disease to AML specifically by inducing differential cytokine expression in the bone marrow microenvironment of MDS-susceptible male mice. Further investigation may allow for novel therapeutics targeting these cytokines or their downstream actions in the bone marrow

Chapter 4: Final Discussion and Future Studies

From our studies, obesity impact on influenza immunization and DKO mice model (myelodysplastic syndrome) has led to a new findings regarding unique cytokine signature and their potential influence on hematopoietic stem and progenitor cells (HSPCs). This, in turn, may affect the immune system or susceptibility to cancer in obese populations.

Previous studies have shown the impact of obesity on influenza immunization in terms of innate and adaptive immune response (Rojas-Osornio et al., 2019), however there is a need to study obesity influence on early stem cells. Cytokines like MIP-1 gamma, IL-3R beta, SCF have shown an increasing trend in high-fat diet flu-immunized mice when compared to low-fat diet flu-immunized mice. These cytokines play a role in stimulating the expansion of HSPCs (Okamatsu et al., 2004) (Hara & Miyajima, 1996) (Li & MacDougald, 2019), which suggests the crucial consequences of increases in these cytokines during obesity-induced inflammation. Increases in these cytokines occur alongside increases in the LSK population in high-fat diet flu-immunized mice, suggesting that obesity-driven alteration in these cytokines may be the mechanism by which LSK frequency increases. Eotaxin significantly increased in the bone marrow of high-fat diet flu-immunized mice. It has a role in stimulating myeloid progenitors and also its quantity increases in influenza-immunization in people with allergic bronchial asthma. Taken together, eotaxin increase in bone marrow may be an underlying mechanism by which

myeloid cells increase in high-fat diet flu-immunized mice. Perhaps it is also a contributor to effects of immunization in people with bronchial asthma.

On the other hand, there were trends of decrease in cytokines like GM-CSF, IL-4, IL-9, MCP-1. GM-CSF and IL-4 in high-fat diet flu-immunized mice. These cytokines are linked with functions in regulating the dynamics of HSPC population, (Sonoda, 1994). IL-9 has its role in inflammation (Matsuzawa et al., 2003). MCP-1 has myelosuppressive effects (Xu et al., 1999), so its decrease in the bone marrow supports our data that shows increased in neutrophils and monocyte proportions in high-fat diet flu-immunized mice. Our blood data show that obesity results in decreased lymphocytes and increased myeloid cells in high-fat diet flu-immunized mice. All in all, our results show that obesity contributes to cytokine alterations which may serve as mechanism to explain the expansion of HSPCs and myeloid skewing following influenza immunization in male mice.

In our second study of obesity, impact on myelodysplastic syndromes (MDS)-susceptible mouse model (DKO), we observed that obesity has sex -specific impacts on weight gain and bone marrow cytokine signatures. Male DKO mice are more susceptible to diet-induced obesity, gaining nearly three times more weight that female mice. Nevertheless, Female mice have shown significant difference in weight on 15 weeks fed with high-fat diet when compared with low-fat diet mice. Cytokine signatures in this model, however, have shown differences among male and female mice, potentially due to increased susceptibility of mice to weight gain. In male high-fat diet flu-immunized mice there is an increase in IL-11 and decreases in HGF and galectin-1. Taken together, these cytokines support proliferation of HSPCs, including megakaryocyte progenitors and decrease in differentiation of HSPCs and

megakaryocyte progenitors which aligns with dysmegakaryopoiesis observed in MDS and acute myeloid leukemia (AML).

Overall, obesity-induced bone marrow cytokine alterations in flu-immunized mice and mice with MDS susceptibility may provide insight as to the mechanisms by which HSPC and immune cell function deviate from that of non-obese populations.

Our future studies regarding immunization include administering stimulators or inhibitors of specific cytokines to obese mice at the time of immunization with influenza vaccine and interpreting the outcomes to determine if this may be a novel therapeutic option. We plan to perform ex vivo cell culture experiments in which HSPCs from low-fat or high-fat diet flu-immunized mice will be cultured bone marrow media with stromal cells. In these experiments, we will add specific cytokines and/or inhibitors of cytokines to determine if manipulation of the cytokine signatures of interest from our studies will lead to altered proliferation and differentiation of HSPCs. In addition to it we also plan to further study female mice resistance to obesity to better understand if there are methods by which cytokine recreating their bone marrow cytokine signatures in males may prevent the effects of obesity on male bone marrow HSPCs.

Future studies regarding our MDS model include treating these MDS-susceptible mice with probiotics to determine if obesity-induced inflammation can be mitigated in a way to restore bone marrow cytokines to levels mimicking non-obese mice. Given that dysbiosis is evident in MDS susceptible mice (Riello et al., 2022), we plan to use *Lactobacillus casei.* It is well-known to exhibit an anti-inflammatory effect (Youssef et al., 2021) Additionally, other studies will include more directly altering cytokines in obese mice with stimulators or

inhibitors of cytokines as appropriate to attempt alter their bone marrow microenvironment to match that of non-obese mice.

References

Adler, B. J., D. E. Green, G. M. Pagnotti, M. E. Chan, and C. T. Rubin. 2014. High fat diet rapidly suppresses B lymphopoiesis by disrupting the supportive capacity of the bone marrow niche, *PLoS One*, 9: e90639.

Adler, B. J., K. Kaushansky, and C. T. Rubin. 2014. Obesity-driven disruption of haematopoiesis and the bone marrow niche, *Nat Rev Endocrinol*, 10: 737-48.

Ali, H., D. Ngo, A. Aribi, S. Arslan, S. Dadwal, G. Marcucci, R. Nakamura, S. J. Forman, J. Chen, and M. M. Al Malki. 2021. Safety and tolerability of SARS-CoV2 emergency-use authorized vaccines for allogeneic hematopoietic stem cell transplant recipients, *Transplant Cell Ther*, 27: 938 e1-38 e6.

Bastidas, A., J. de la Serna, M. El Idrissi, L. Oostvogels, P. Quittet, J. Lopez-Jimenez, F. Vural, D. Pohlreich, T. Zuckerman, N. C. Issa, G. Gaidano, J. J. Lee, S. Abhyankar, C. Solano, J. Perez de Oteyza, M. J. Satlin, S. Schwartz, M. Campins, A. Rocci, C. Vallejo Llamas, D. G. Lee, S. M. Tan, A. M. Johnston, A. Grigg, M. J. Boeckh, L. Campora, M. Lopez-Fauqued, T. C. Heineman, E. A. Stadtmauer, K. M. Sullivan, and Zoe-Hsct Study Group Collaborators. 2019. Effect of recombinant zoster vaccine on incidence of herpes zoster after autologous stem cell transplantation: A randomized clinical trial, *JAMA*, 322: 123-33.

Benova, A., and M. Tencerova. 2020. Obesity-induced changes in bone marrow homeostasis, *Front Endocrinol (Lausanne)*, 11: 294.

Bilwani, F. A., and K. L. Knight. 2012. Adipocyte-derived soluble factor(s) inhibits early stages of B lymphopoiesis, *J Immunol*, 189: 4379-86.

Chan, M. E., B. J. Adler, D. E. Green, and C. T. Rubin. 2012. Bone structure and B-cell populations, crippled by obesity, are partially rescued by brief daily exposure to low-magnitude mechanical signals, *FASEB J*, 26: 4855-63.

Clinckaert, A., K. Callens, A. Cooreman, A. Bijnens, L. Moris, C. Van Calster, I. Geraerts, S. Joniau, and W. Everaerts. 2022. The prevalence of lower limb and genital lymphedema after prostate cancer treatment: A systematic review, *Cancers (Basel)*, 14.

Cordonnier, C., S. Einarsdottir, S. Cesaro, R. Di Blasi, M. Mikulska, C. Rieger, H. de Lavallade, G. Gallo, T. Lehrnbecher, D. Engelhard, P. Ljungman, and group European Conference on Infections in Leukaemia. 2019. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European Conference on Infections in Leukaemia (ECIL 7), *Lancet Infect Dis*, 19: e200-e12.

Emmons, R., G. M. Niemiro, and M. De Lisio. 2017. Hematopoiesis with obesity and exercise: Role of the bone marrow niche, *Exerc Immunol Rev*, 23: 82-95.

Fried, S. K., D. A. Bunkin, and A. S. Greenberg. 1998. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoid, *J Clin Endocrinol Metab*, 83: 847-50.

Fukuhara, A., M. Matsuda, M. Nishizawa, K. Segawa, M. Tanaka, K. Kishimoto, Y. Matsuki, M. Murakami, T. Ichisaka, H. Murakami, E. Watanabe, T. Takagi, M. Akiyoshi, T. Ohtsubo, S. Kihara, S. Yamashita, M. Makishima, T. Funahashi, S. Yamanaka, R. Hiramatsu, Y. Matsuzawa, and I. Shimomura. 2005. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin, *Science*, 307: 426-30.

Geyh, S., S. Oz, R. P. Cadeddu, J. Frobel, B. Bruckner, A. Kundgen, R. Fenk, I. Bruns, C. Zilkens, D. Hermsen, N. Gattermann, G. Kobbe, U. Germing, F. Lyko, R. Haas, and T. Schroeder.

2013. Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells, *Leukemia*, 27: 1841-51.

Greenbaum, A., Y. M. Hsu, R. B. Day, L. G. Schuettpelz, M. J. Christopher, J. N. Borgerding, T. Nagasawa, and D. C. Link. 2013. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance, *Nature*, 495: 227-30.

Gregor, M. F., and G. S. Hotamisligil. 2011. Inflammatory mechanisms in obesity, *Annu Rev Immunol*, 29: 415-45.

Habanjar, O., M. Diab-Assaf, F. Caldefie-Chezet, and L. Delort. 2022. The impact of obesity, adipose tissue, and tumor microenvironment on macrophage polarization and Metastasis', *Biology (Basel)*, 11.

Hirosumi, J., G. Tuncman, L. Chang, C. Z. Gorgun, K. T. Uysal, K. Maeda, M. Karin, and G. S. Hotamisligil. 2002. A central role for JNK in obesity and insulin resistance, *Nature*, 420: 333-6.

Hotamisligil, G. S., P. Arner, J. F. Caro, R. L. Atkinson, and B. M. Spiegelman. 1995. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance, *J Clin Invest*, 95: 2409-15.

Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance, *Science*, 259: 87-91.

Hou, J., C. He, W. He, M. Yang, X. Luo, and C. Li. 2020. Obesity and bone health: A complex link, *Front Cell Dev Biol*, 8: 600181.

Kaufmann, E., J. Sanz, J. L. Dunn, N. Khan, L. E. Mendonca, A. Pacis, F. Tzelepis, E. Pernet, A. Dumaine, J. C. Grenier, F. Mailhot-Leonard, E. Ahmed, J. Belle, R. Besla, B. Mazer, I. L. King, A. Nijnik, C. S. Robbins, L. B. Barreiro, and M. Divangahi. 2018. BCG educates

hematopoietic stem cells to generate protective innate immunity against tuberculosis, *Cell*, 172: 176-90 e19.

Mattiucci, D., G. Maurizi, V. Izzi, L. Cenci, M. Ciarlantini, S. Mancini, E. Mensa, R. Pascarella, M. Vivarelli, A. Olivieri, P. Leoni, and A. Poloni. 2018. Bone marrow adipocytes support hematopoietic stem cell survival, *J Cell Physiol*, 233: 1500-11.

Raaijmakers, M. H. 2012. Myelodysplastic syndromes: revisiting the role of the bone marrow microenvironment in disease pathogenesis', *Int J Hematol*, 95: 17-25.

Reagan, M. R., H. Fairfield, and C. J. Rosen. 2021. Bone marrow adipocytes: A link between obesity and bone cancer, *Cancers (Basel)*, 13.

Sallman, D. A., and A. List. 2019. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes, *Blood*, 133: 1039-48.

Selleri, C., T. Sato, S. Anderson, N. S. Young, and J. P. Maciejewski. 1995. Interferon-gamma and tumor necrosis factor-alpha suppress both early and late stages of hematopoiesis and induce programmed cell death, *J Cell Physiol*, 165: 538-46.

Shimomura, I., T. Funahashi, M. Takahashi, K. Maeda, K. Kotani, T. Nakamura, S. Yamashita, M. Miura, Y. Fukuda, K. Takemura, K. Tokunaga, and Y. Matsuzawa. 1996. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity, *Nat Med*, 2: 800-3.

Shoelson, S. E., J. Lee, and A. B. Goldfine. 2006. Inflammation and insulin resistance, *J Clin Invest*, 116: 1793-801.

Solinas, G., and M. Karin. 2010. JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction, *FASEB J*, 24: 2596-611.

Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima, and M. A. Lazar. 2001. The hormone resistin links obesity to diabetes, *Nature*, 409: 307-12.

Suresh, S., J. C. Alvarez, S. Dey, and C. T. Noguchi. 2020. Erythropoietin-induced changes in bone and bone marrow in mouse models of diet-induced obesity, *Int J Mol Sci*, 21.

Tencerova, M., M. Okla, and M. Kassem. 2019. Insulin Signaling in Bone Marrow Adipocytes, *Curr Osteoporos Rep*, 17: 446-54.

Yamashita, M., and E. Passegue. 2019. TNF-alpha coordinates hematopoietic stem cell survival and myeloid regeneration, *Cell Stem Cell*, 25: 357-72 e7.

Zhou, B. O., H. Yu, R. Yue, Z. Zhao, J. J. Rios, O. Naveiras, and S. J. Morrison. 2017. Bone marrow adipocytes promote the regeneration of stem cells and haematopoiesis by secreting SCF, *Nat Cell Biol*, 19: 891-903.

Adler, B. J., Green, D. E., Pagnotti, G. M., Chan, M. E., & Rubin, C. T. (2014). High fat diet rapidly suppresses B lymphopoiesis by disrupting the supportive capacity of the bone marrow niche. *PLoS One, 9*(3), e90639. https://doi.org/10.1371/journal.pone.0090639

Adler, B. J., Kaushansky, K., & Rubin, C. T. (2014, Dec). Obesity-driven disruption of haematopoiesis and the bone marrow niche. *Nat Rev Endocrinol, 10*(12), 737-748. https://doi.org/10.1038/nrendo.2014.169

Al-Alwan, L. A., Chang, Y., Mogas, A., Halayko, A. J., Baglole, C. J., Martin, J. G., Rousseau, S., Eidelman, D. H., & Hamid, Q. (2013, Sep 1). Differential roles of CXCL2 and CXCL3 and their receptors in regulating normal and asthmatic airway smooth muscle cell migration. *J Immunol, 191*(5), 2731-2741. https://doi.org/10.4049/jimmunol.1203421

Almond, M. H., Edwards, M. R., Barclay, W. S., & Johnston, S. L. (2013, Jul). Obesity and susceptibility to severe outcomes following respiratory viral infection. *Thorax, 68*(7), 684-686. https://doi.org/10.1136/thoraxjnl-2012-203009

Angulo-Zamudio, U. A., Martinez-Villa, F. M., Leon-Sicairos, N., Flores-Villasenor, H., Velazquez-Roman, J., Campos-Romero, A., Alcantar-Fernandez, J., Urrea, F., Muro-Amador, S., Medina-Serrano, J., Martinez-Garcia, J. J., Sanchez-Cuen, J., Angulo-Rocha, J., & Canizalez-Roman, A. (2021). Analysis of epidemiological and clinical characteristics of COVID-19 in northwest mexico and the relationship between the influenza vaccine and the survival of infected patients. *Front Public Health, 9*, 570098. https://doi.org/10.3389/fpubh.2021.570098

Apovian, C. M. (2016, Jun). Obesity: definition, comorbidities, causes, and burden. *Am J Manag Care, 22*(7 Suppl), s176-185. https://www.ncbi.nlm.nih.gov/pubmed/27356115

Avgerinos, K. I., Spyrou, N., Mantzoros, C. S., & Dalamaga, M. (2019, Mar). Obesity and cancer risk: Emerging biological mechanisms and perspectives. *Metabolism, 92*, 121-135. https://doi.org/10.1016/j.metabol.2018.11.001

Barreyro, L., Chlon, T. M., & Starczynowski, D. T. (2018, Oct 11). Chronic immune response dysregulation in MDS pathogenesis. *Blood, 132*(15), 1553-1560. https://doi.org/10.1182/blood-2018-03-784116

Benova, A., & Tencerova, M. (2020). Obesity-Induced changes in bone marrow homeostasis. *Front Endocrinol (Lausanne), 11*, 294. https://doi.org/10.3389/fendo.2020.00294

Bilwani, F. A., & Knight, K. L. (2012, Nov 1). Adipocyte-derived soluble factor(s) inhibits early stages of B lymphopoiesis. *J Immunol, 189*(9), 4379-4386. https://doi.org/10.4049/jimmunol.1201176

Boultwood, J., Pellagatti, A., Cattan, H., Lawrie, C. H., Giagounidis, A., Malcovati, L., Della Porta, M. G., Jadersten, M., Killick, S., Fidler, C., Cazzola, M., Hellstrom-Lindberg, E., & Wainscoat, J. S. (2007, Nov). Gene expression profiling of CD34+ cells in patients with the 5q- syndrome. *Br J Haematol, 139*(4), 578-589. https://doi.org/10.1111/j.1365-2141.2007.06833.x

Cai, Z., Greene, M. I., Zhu, Z., & Zhang, H. (2020, Oct 10). Structural features and PF4 functions that occur in heparin-induced thrombocytopenia (HIT) complicated by COVID-19. *Antibodies (Basel), 9*(4). https://doi.org/10.3390/antib9040052

Calle, E. E., Rodriguez, C., Walker-Thurmond, K., & Thun, M. J. (2003, Apr 24). Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med, 348*(17), 1625-1638. https://doi.org/10.1056/NEJMoa021423

Carr, M. W., Roth, S. J., Luther, E., Rose, S. S., & Springer, T. A. (1994, Apr 26). Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc Natl Acad Sci U S A, 91*(9), 3652-3656. https://doi.org/10.1073/pnas.91.9.3652

Chan, M. E., Adler, B. J., Green, D. E., & Rubin, C. T. (2012, Dec). Bone structure and B-cell populations, crippled by obesity, are partially rescued by brief daily exposure to low-magnitude mechanical signals. *FASEB J, 26*(12), 4855-4863. https://doi.org/10.1096/fj.12-209841

Cho, W. J., Lee, D. K., Lee, S. Y., Sohn, S. H., Park, H. L., Park, Y. W., Kim, H., & Nam, J. H. (2016). Diet-induced obesity reduces the production of influenza vaccine-induced antibodies via impaired macrophage function. *Acta Virol, 60*(3), 298-306. https://doi.org/10.4149/av_2016_03_298

Clinckaert, A., Callens, K., Cooreman, A., Bijnens, A., Moris, L., Van Calster, C., Geraerts, I., Joniau, S., & Everaerts, W. (2022, Nov 18). The prevalence of lower limb and genital lymphedema after prostate cancer treatment: A systematic review. *Cancers (Basel), 14*(22). https://doi.org/10.3390/cancers14225667

de Frel, D. L., Atsma, D. E., Pijl, H., Seidell, J. C., Leenen, P. J. M., Dik, W. A., & van Rossum, E. F. C. (2020). The impact of obesity and lifestyle on the immune system and susceptibility to infections such as COVID-19. *Front Nutr, 7*, 597600. https://doi.org/10.3389/fnut.2020.597600

De Paoli, M., Zakharia, A., & Werstuck, G. H. (2021, Sep). The Role of estrogen in insulin resistance: A review of clinical and preclinical data. *Am J Pathol, 191*(9), 1490-1498. https://doi.org/10.1016/j.ajpath.2021.05.011

Deotare, U., Al-Dawsari, G., Couban, S., & Lipton, J. H. (2015, Sep). G-CSF-primed bone marrow as a source of stem cells for allografting: revisiting the concept. *Bone Marrow Transplant, 50*(9), 1150-1156. https://doi.org/10.1038/bmt.2015.80

Devalaraja, R. M., Nanney, L. B., Du, J., Qian, Q., Yu, Y., Devalaraja, M. N., & Richmond, A. (2000, Aug). Delayed wound healing in CXCR2 knockout mice. *J Invest Dermatol, 115*(2), 234-244. https://doi.org/10.1046/j.1523-1747.2000.00034.x

Du, X. X., & Williams, D. A. (1994, Apr 15). Interleukin-11: a multifunctional growth factor derived from the hematopoietic microenvironment. *Blood, 83*(8), 2023-2030. https://www.ncbi.nlm.nih.gov/pubmed/7512836

Emmons, R., Niemiro, G. M., & De Lisio, M. (2017). Hematopoiesis with obesity and exercise: Role of the bone marrow niche. *Exerc Immunol Rev, 23*, 82-95. https://www.ncbi.nlm.nih.gov/pubmed/28224968

Evers, T. M. J., Sheikhhassani, V., Haks, M. C., Storm, C., Ottenhoff, T. H. M., & Mashaghi, A. (2022, Jan 21). Single-cell analysis reveals chemokine-mediated differential regulation of monocyte mechanics. *iScience, 25*(1), 103555. https://doi.org/10.1016/j.isci.2021.103555

Falconi, G., Fabiani, E., Criscuolo, M., Fianchi, L., Finelli, C., Cerqui, E., Pelosi, E., Screnci, M., Gurnari, C., Zangrilli, I., Postorino, M., Laurenti, L., Piciocchi, A., Testa, U., Lo-Coco, F., & Voso, M. T. (2019, Sep). Transcription factors implicated in late megakaryopoiesis as markers of outcome after azacitidine and allogeneic stem cell transplantation in myelodysplastic syndrome. *Leuk Res, 84*, 106191. https://doi.org/10.1016/j.leukres.2019.106191

Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998, Mar). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab, 83*(3), 847-850. https://doi.org/10.1210/jcem.83.3.4660

Fukuhara, A., Matsuda, M., Nishizawa, M., Segawa, K., Tanaka, M., Kishimoto, K., Matsuki, Y., Murakami, M., Ichisaka, T., Murakami, H., Watanabe, E., Takagi, T., Akiyoshi, M., Ohtsubo, T., Kihara, S., Yamashita, S., Makishima, M., Funahashi, T., Yamanaka, S.,

Hiramatsu, R., Matsuzawa, Y., & Shimomura, I. (2005, Jan 21). Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science, 307*(5708), 426-430. https://doi.org/10.1126/science.1097243

Geyh, S., Oz, S., Cadeddu, R. P., Frobel, J., Bruckner, B., Kundgen, A., Fenk, R., Bruns, I., Zilkens, C., Hermsen, D., Gattermann, N., Kobbe, G., Germing, U., Lyko, F., Haas, R., & Schroeder, T. (2013, Sep). Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. *Leukemia, 27*(9), 1841-1851. https://doi.org/10.1038/leu.2013.193

Greenbaum, A., Hsu, Y. M., Day, R. B., Schuettpelz, L. G., Christopher, M. J., Borgerding, J. N., Nagasawa, T., & Link, D. C. (2013, Mar 14). CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature, 495*(7440), 227-230. https://doi.org/10.1038/nature11926

Gregor, M. F., & Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annu Rev Immunol, 29*, 415-445. https://doi.org/10.1146/annurev-immunol-031210-101322

Habanjar, O., Diab-Assaf, M., Caldefie-Chezet, F., & Delort, L. (2022, Feb 21). The impact of obesity, adipose tissue, and tumor microenvironment on macrophage polarization and metastasis. *Biology (Basel), 11*(2). https://doi.org/10.3390/biology11020339

Hara, T., & Miyajima, A. (1996, Nov). Function and signal transduction mediated by the interleukin 3 receptor system in hematopoiesis. *Stem Cells, 14*(6), 605-618. https://doi.org/10.1002/stem.140605

Hercus, T. R., Thomas, D., Guthridge, M. A., Ekert, P. G., King-Scott, J., Parker, M. W., & Lopez, A. F. (2009, Aug 13). The granulocyte-macrophage colony-stimulating factor

receptor: linking its structure to cell signaling and its role in disease. *Blood, 114*(7), 1289-1298. https://doi.org/10.1182/blood-2008-12-164004

Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C. Z., Uysal, K. T., Maeda, K., Karin, M., & Hotamisligil, G. S. (2002, Nov 21). A central role for JNK in obesity and insulin resistance. *Nature, 420*(6913), 333-336. https://doi.org/10.1038/nature01137

Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L., & Spiegelman, B. M. (1995, May). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest, 95*(5), 2409-2415. https://doi.org/10.1172/JCI117936

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993, Jan 1). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science, 259*(5091), 87-91. https://doi.org/10.1126/science.7678183

Hou, J., He, C., He, W., Yang, M., Luo, X., & Li, C. (2020). Obesity and bone health: A complex link. *Front Cell Dev Biol, 8*, 600181. https://doi.org/10.3389/fcell.2020.600181

Ikehara, S. (1996, Oct). Role of hepatocyte growth factor in hemopoiesis. *Leuk Lymphoma, 23*(3-4), 297-303. https://doi.org/10.3109/10428199609054832

Jahnz-Rozyk, K., Brydak, L. B., Targowski, T., Machala, M., & Plusa, T. (2004, Jun). Effect of influenza vaccinations on immune response and serum eotaxin level in patients with allergic bronchial asthma. *Mediators Inflamm, 13*(3), 195-199. https://doi.org/10.1080/09511920410001713501

Jerez, A., Gondek, L. P., Jankowska, A. M., Makishima, H., Przychodzen, B., Tiu, R. V., O'Keefe, C. L., Mohamedali, A. M., Batista, D., Sekeres, M. A., McDevitt, M. A., Mufti,

G. J., & Maciejewski, J. P. (2012, Apr 20). Topography, clinical, and genomic correlates of 5q myeloid malignancies revisited. *J Clin Oncol, 30*(12), 1343-1349. https://doi.org/10.1200/JCO.2011.36.1824

Kaufmann, E., Sanz, J., Dunn, J. L., Khan, N., Mendonca, L. E., Pacis, A., Tzelepis, F., Pernet, E., Dumaine, A., Grenier, J. C., Mailhot-Leonard, F., Ahmed, E., Belle, J., Besla, R., Mazer, B., King, I. L., Nijnik, A., Robbins, C. S., Barreiro, L. B., & Divangahi, M. (2018, Jan 11). BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell, 172*(1-2), 176-190 e119. https://doi.org/10.1016/j.cell.2017.12.031

Keller, U., Aman, M. J., Derigs, G., Huber, C., & Peschel, C. (1994, Oct 1). Human interleukin-4 enhances stromal cell-dependent hematopoiesis: costimulation with stem cell factor. *Blood, 84*(7), 2189-2196. https://www.ncbi.nlm.nih.gov/pubmed/7522623

Kim, Y. H., Kim, J. K., Kim, D. J., Nam, J. H., Shim, S. M., Choi, Y. K., Lee, C. H., & Poo, H. (2012, Jan 15). Diet-induced obesity dramatically reduces the efficacy of a 2009 pandemic H1N1 vaccine in a mouse model. *J Infect Dis, 205*(2), 244-251. https://doi.org/10.1093/infdis/jir731

Lagergren, A., Mansson, R., Zetterblad, J., Smith, E., Basta, B., Bryder, D., Akerblad, P., &Sigvardsson, M. (2007, May 11). The Cxcl12, periostin, and Ccl9 genes are direct targets for early B-cell factor in OP-9 stroma cells. *J Biol Chem, 282*(19), 14454-14462. https://doi.org/10.1074/jbc.M610263200

Li, Z., & MacDougald, O. A. (2019, Sep). Stem cell factor: the bridge between bone marrow adipocytes and hematopoietic cells. *Haematologica, 104*(9), 1689-1691. https://doi.org/10.3324/haematol.2019.224188

Liu, M., Guo, S., Hibbert, J. M., Jain, V., Singh, N., Wilson, N. O., & Stiles, J. K. (2011, Jun). CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. *Cytokine Growth Factor Rev, 22*(3), 121-130. https://doi.org/10.1016/j.cytogfr.2011.06.001

Lopez-Castejon, G., & Brough, D. (2011, Aug). Understanding the mechanism of IL-1beta secretion. *Cytokine Growth Factor Rev, 22*(4), 189-195. https://doi.org/10.1016/j.cytogfr.2011.10.001

Ma, X., Lim, U., Park, Y., Mayne, S. T., Wang, R., Hartge, P., Hollenbeck, A. R., & Schatzkin, A. (2009, Jun 15). Obesity, lifestyle factors, and risk of myelodysplastic syndromes in a large US cohort. *Am J Epidemiol, 169*(12), 1492-1499. https://doi.org/10.1093/aje/kwp074

Maggio, C. A., & Pi-Sunyer, F. X. (2003, Dec). Obesity and type 2 diabetes. *Endocrinol Metab Clin North Am, 32*(4), 805-822, viii. https://doi.org/10.1016/s0889-8529(03)00071-9

Maltezou, H. C., Theodoridou, K., Ledda, C., Rapisarda, V., & Theodoridou, M. (2019, Jan). Vaccination of healthcare workers: is mandatory vaccination needed? *Expert Rev Vaccines, 18*(1), 5-13. https://doi.org/10.1080/14760584.2019.1552141

Marshall, S. J. (2004, Jul). Developing countries face double burden of disease. *Bull World Health Organ, 82*(7), 556. https://www.ncbi.nlm.nih.gov/pubmed/15500291

Matsuzawa, S., Sakashita, K., Kinoshita, T., Ito, S., Yamashita, T., & Koike, K. (2003, Apr 1). IL-9 enhances the growth of human mast cell progenitors under stimulation with stem cell factor. *J Immunol, 170*(7), 3461-3467. https://doi.org/10.4049/jimmunol.170.7.3461

Mattiucci, D., Maurizi, G., Izzi, V., Cenci, L., Ciarlantini, M., Mancini, S., Mensa, E., Pascarella, R., Vivarelli, M., Olivieri, A., Leoni, P., & Poloni, A. (2018, Feb). Bone marrow adipocytes support hematopoietic stem cell survival. *J Cell Physiol, 233*(2), 1500-1511. https://doi.org/10.1002/jcp.26037

Mayani, H., Guilbert, L. J., & Janowska-Wieczorek, A. (1992, Nov). Biology of the hemopoietic microenvironment. *Eur J Haematol, 49*(5), 225-233. https://doi.org/10.1111/j.1600-0609.1992.tb00053.x

Memari, B., Bouttier, M., Dimitrov, V., Ouellette, M., Behr, M. A., Fritz, J. H., & White, J. H. (2015, Nov 1). Engagement of the aryl hydrocarbon receptor in mycobacterium tuberculosis-Infected macrophages has pleiotropic effects on innate immune signaling. *J Immunol, 195*(9), 4479-4491. https://doi.org/10.4049/jimmunol.1501141

Michailidou, Z., Gomez-Salazar, M., & Alexaki, V. I. (2022). Innate immune cells in the adipose tissue in health and metabolic disease. *J Innate Immun, 14*(1), 4-30. https://doi.org/10.1159/000515117

Milner, J. J., Rebeles, J., Dhungana, S., Stewart, D. A., Sumner, S. C., Meyers, M. H., Mancuso, P., & Beck, M. A. (2015, May 15). Obesity increases mortality and modulates the lung metabolome during pandemic H1N1 influenza virus infection in mice. *J Immunol, 194*(10), 4846-4859. https://doi.org/10.4049/jimmunol.1402295

Mitroulis, I., Ruppova, K., Wang, B., Chen, L. S., Grzybek, M., Grinenko, T., Eugster, A., Troullinaki, M., Palladini, A., Kourtzelis, I., Chatzigeorgiou, A., Schlitzer, A., Beyer, M., Joosten, L. A. B., Isermann, B., Lesche, M., Petzold, A., Simons, K., Henry, I., Dahl, A., Schultze, J. L., Wielockx, B., Zamboni, N., Mirtschink, P., Coskun, U., Hajishengallis, G., Netea, M. G., & Chavakis, T. (2018, Jan 11). Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell, 172*(1-2), 147-161 e112. https://doi.org/10.1016/j.cell.2017.11.034

Mohamadzadeh, M., Poltorak, A. N., Bergstressor, P. R., Beutler, B., & Takashima, A. (1996, May 1). Dendritic cells produce macrophage inflammatory protein-1 gamma, a new member of the CC chemokine family. *J Immunol, 156*(9), 3102-3106. https://www.ncbi.nlm.nih.gov/pubmed/8617929

Monteiro, R., & Azevedo, I. (2010). Chronic inflammation in obesity and the metabolic syndrome. *Mediators Inflamm, 2010*. https://doi.org/10.1155/2010/289645

Murphy, F., Kroll, M. E., Pirie, K., Reeves, G., Green, J., & Beral, V. (2013, Jun 11). Body size in relation to incidence of subtypes of haematological malignancy in the prospective Million Women Study. *Br J Cancer, 108*(11), 2390-2398. https://doi.org/10.1038/bjc.2013.159

Nagareddy, P. R., Kraakman, M., Masters, S. L., Stirzaker, R. A., Gorman, D. J., Grant, R. W., Dragoljevic, D., Hong, E. S., Abdel-Latif, A., Smyth, S. S., Choi, S. H., Korner, J., Bornfeldt, K. E., Fisher, E. A., Dixit, V. D., Tall, A. R., Goldberg, I. J., & Murphy, A. J. (2014, May 6). Adipose tissue macrophages promote myelopoiesis and monocytosis in obesity. *Cell Metab, 19*(5), 821-835. https://doi.org/10.1016/j.cmet.2014.03.029

Naveiras, O., Nardi, V., Wenzel, P. L., Hauschka, P. V., Fahey, F., & Daley, G. Q. (2009, Jul 9). Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature, 460*(7252), 259-263. https://doi.org/10.1038/nature08099

Okamatsu, Y., Kim, D., Battaglino, R., Sasaki, H., Spate, U., & Stashenko, P. (2004, Aug 1). MIP-1 gamma promotes receptor-activator-of-NF-kappa-B-ligand-induced osteoclast formation and survival. *J Immunol, 173*(3), 2084-2090. https://doi.org/10.4049/jimmunol.173.3.2084

Orgel, E., Genkinger, J. M., Aggarwal, D., Sung, L., Nieder, M., & Ladas, E. J. (2016, Mar). Association of body mass index and survival in pediatric leukemia: a meta-analysis. *Am J Clin Nutr, 103*(3), 808-817. https://doi.org/10.3945/ajcn.115.124586

Powell-Wiley, T. M., Poirier, P., Burke, L. E., Despres, J. P., Gordon-Larsen, P., Lavie, C. J., Lear, S. A., Ndumele, C. E., Neeland, I. J., Sanders, P., St-Onge, M. P., American Heart Association Council on, L., Cardiometabolic, H., Council on, C., Stroke, N., Council on Clinical, C., Council on, E., Prevention, & Stroke, C. (2021, May 25). Obesity and cardiovascular disease: A scientific statement from the american heart association. *Circulation, 143*(21), e984-e1010. https://doi.org/10.1161/CIR.0000000000000973

Raaijmakers, M. H. (2012, Jan). Myelodysplastic syndromes: revisiting the role of the bone marrow microenvironment in disease pathogenesis. *Int J Hematol, 95*(1), 17-25. https://doi.org/10.1007/s12185-011-1001-x

Rabinovich, G. A., & Vidal, M. (2011, Nov). Galectins and microenvironmental niches during hematopoiesis. *Curr Opin Hematol, 18*(6), 443-451. https://doi.org/10.1097/MOH.0b013e32834bab18

Radinger, M., Johansson, A. K., Sitkauskiene, B., Sjostrand, M., & Lotvall, J. (2004, Jun). Eotaxin-2 regulates newly produced and CD34 airway eosinophils after allergen exposure. *J Allergy Clin Immunol, 113*(6), 1109-1116. https://doi.org/10.1016/j.jaci.2004.03.022

Rasheed, A. (2022, Jun). Niche regulation of hematopoiesis: The environment is "micro," but the influence is large. *Arterioscler Thromb Vasc Biol, 42*(6), 691-699. https://doi.org/10.1161/ATVBAHA.121.316235

Reagan, M. R., Fairfield, H., & Rosen, C. J. (2021, Jan 20). Bone marrow adipocytes: A link between obesity and bone cancer. *Cancers (Basel), 13*(3). https://doi.org/10.3390/cancers13030364

Rojas-Osornio, S. A., Cruz-Hernandez, T. R., Drago-Serrano, M. E., & Campos-Rodriguez, R. (2019, Sep-Oct). Immunity to influenza: Impact of obesity. *Obes Res Clin Pract, 13*(5), 419-429. https://doi.org/10.1016/j.orcp.2019.05.003

Sallman, D. A., & List, A. (2019, Mar 7). The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. *Blood, 133*(10), 1039-1048. https://doi.org/10.1182/blood-2018-10-844654

Selleri, C., Sato, T., Anderson, S., Young, N. S., & Maciejewski, J. P. (1995, Dec). Interferon-gamma and tumor necrosis factor-alpha suppress both early and late stages of hematopoiesis and induce programmed cell death. *J Cell Physiol, 165*(3), 538-546. https://doi.org/10.1002/jcp.1041650312

Sherry, B., Tekamp-Olson, P., Gallegos, C., Bauer, D., Davatelis, G., Wolpe, S. D., Masiarz, F., Coit, D., & Cerami, A. (1988, Dec 1). Resolution of the two components of macrophage

inflammatory protein 1, and cloning and characterization of one of those components, macrophage inflammatory protein 1 beta. *J Exp Med, 168*(6), 2251-2259. https://doi.org/10.1084/jem.168.6.2251

Shimomura, I., Funahashi, T., Takahashi, M., Maeda, K., Kotani, K., Nakamura, T., Yamashita, S., Miura, M., Fukuda, Y., Takemura, K., Tokunaga, K., & Matsuzawa, Y. (1996, Jul). Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med, 2*(7), 800-803. https://doi.org/10.1038/nm0796-800

Shoelson, S. E., Lee, J., & Goldfine, A. B. (2006, Jul). Inflammation and insulin resistance. *J Clin Invest, 116*(7), 1793-1801. https://doi.org/10.1172/JCI29069

Siffert, R. S. (1987, Sep). Lower limb-length discrepancy. *J Bone Joint Surg Am, 69*(7), 1100-1106. https://www.ncbi.nlm.nih.gov/pubmed/3308894

Silva, R. L., Lopes, A. H., Guimaraes, R. M., & Cunha, T. M. (2017, Sep). CXCL1/CXCR2 signaling in pathological pain: Role in peripheral and central sensitization. *Neurobiol Dis, 105*, 109-116. https://doi.org/10.1016/j.nbd.2017.06.001

Sindhu, S., Kochumon, S., Shenouda, S., Wilson, A., Al-Mulla, F., & Ahmad, R. (2019, Sep 19). The cooperative induction of CCL4 in human monocytic cells by TNF-alpha and palmitate requires MyD88 and involves MAPK/NF-kappaB signaling pathways. *Int J Mol Sci, 20*(18). https://doi.org/10.3390/ijms20184658

Solinas, G., & Karin, M. (2010, Aug). JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction. *FASEB J, 24*(8), 2596-2611. https://doi.org/10.1096/fj.09-151340

Sonoda, Y. (1994, Jul). Interleukin-4--a dual regulatory factor in hematopoiesis. *Leuk Lymphoma, 14*(3-4), 231-240. https://doi.org/10.3109/10428199409049673

Starczynowski, D. T. (2014, Jul 31). Errant innate immune signaling in del(5q) MDS. *Blood, 124*(5), 669-671. https://doi.org/10.1182/blood-2014-06-581728

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., Patel, H. R., Ahima, R. S., & Lazar, M. A. (2001, Jan 18). The hormone resistin links obesity to diabetes. *Nature, 409*(6818), 307-312. https://doi.org/10.1038/35053000

Suresh, S., Alvarez, J. C., Dey, S., & Noguchi, C. T. (2020, Feb 28). Erythropoietin-Induced changes in bone and bone marrow in mouse models of diet-induced obesity. *Int J Mol Sci, 21*(5). https://doi.org/10.3390/ijms21051657

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014, Sep 4). IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol, 6*(10), a016295. https://doi.org/10.1101/cshperspect.a016295

Tay, J., Levesque, J. P., & Winkler, I. G. (2017, Feb). Cellular players of hematopoietic stem cell mobilization in the bone marrow niche. *Int J Hematol, 105*(2), 129-140. https://doi.org/10.1007/s12185-016-2162-4

Tehranchi, R., Woll, P. S., Anderson, K., Buza-Vidas, N., Mizukami, T., Mead, A. J., Astrand-Grundstrom, I., Strombeck, B., Horvat, A., Ferry, H., Dhanda, R. S., Hast, R., Ryden, T., Vyas, P., Gohring, G., Schlegelberger, B., Johansson, B., Hellstrom-Lindberg, E., List, A., Nilsson, L., & Jacobsen, S. E. (2010, Sep 9). Persistent malignant stem cells in del(5q) myelodysplasia in remission. *N Engl J Med, 363*(11), 1025-1037. https://doi.org/10.1056/NEJMoa0912228

Tencerova, M., Okla, M., & Kassem, M. (2019, Dec). Insulin signaling in bone marrow adipocytes. *Curr Osteoporos Rep, 17*(6), 446-454. https://doi.org/10.1007/s11914-019-00552-8

Tumminia, A., Vinciguerra, F., Parisi, M., Graziano, M., Sciacca, L., Baratta, R., & Frittitta, L. (2019, Jun 12). Adipose tissue, obesity and adiponectin: Role in endocrine cancer risk. *Int J Mol Sci, 20*(12). https://doi.org/10.3390/ijms20122863

Turner, K. J., Neben, S., Weich, N., Schaub, R. G., & Goldman, S. J. (1996). The role of recombinant interleukin 11 in megakaryocytopoiesis. *Stem Cells, 14 Suppl 1*, 53-61. https://doi.org/10.1002/stem.5530140707

van den Berg, S. M., Seijkens, T. T., Kusters, P. J., Beckers, L., den Toom, M., Smeets, E., Levels, J., de Winther, M. P., & Lutgens, E. (2016, May). Diet-induced obesity in mice diminishes hematopoietic stem and progenitor cells in the bone marrow. *FASEB J, 30*(5), 1779-1788. https://doi.org/10.1096/fj.201500175

Varney, M. E., Boehm, D. T., DeRoos, K., Nowak, E. S., Wong, T. Y., Sen-Kilic, E., Bradford, S. D., Elkins, C., Epperly, M. S., Witt, W. T., Barbier, M., & Damron, F. H. (2018). Bordetella pertussis whole cell immunization, unlike acellular immunization, mimics naive infection by driving hematopoietic stem and progenitor cell expansion in mice. *Front Immunol, 9*, 2376. https://doi.org/10.3389/fimmu.2018.02376

Varney, M. E., Melgar, K., Niederkorn, M., Smith, M., Barreyro, L., & Starczynowski, D. T. (2015, Aug). Deconstructing innate immune signaling in myelodysplastic syndromes. *Exp Hematol, 43*(8), 587-598. https://doi.org/10.1016/j.exphem.2015.05.016

Varney, M. E., Niederkorn, M., Konno, H., Matsumura, T., Gohda, J., Yoshida, N., Akiyama, T., Christie, S., Fang, J., Miller, D., Jerez, A., Karsan, A., Maciejewski, J. P., Meetei, R. A., Inoue, J., & Starczynowski, D. T. (2015, Oct 19). Loss of Tifab, a del(5q) MDS gene, alters hematopoiesis through derepression of Toll-like receptor-TRAF6 signaling. *J Exp Med, 212*(11), 1967-1985. https://doi.org/10.1084/jem.20141898

Vas, V., Fajka-Boja, R., Ion, G., Dudics, V., Monostori, E., & Uher, F. (2005, Feb). Biphasic effect of recombinant galectin-1 on the growth and death of early hematopoietic cells. *Stem Cells, 23*(2), 279-287. https://doi.org/10.1634/stemcells.2004-0084

Vasseur, F., Le Campion, A., Pavlovitch, J. H., & Penit, C. (1999, May 1). Distribution of cycling T lymphocytes in blood and lymphoid organs during immune responses. *J Immunol, 162*(9), 5164-5172. https://www.ncbi.nlm.nih.gov/pubmed/10227988

Wahl, C., Miethke, T., Heeg, K., & Wagner, H. (1993, May). Clonal deletion as direct consequence of an in vivo T cell response to bacterial superantigen. *Eur J Immunol, 23*(5), 1197-1200. https://doi.org/10.1002/eji.1830230536

Xing, T., Lyu, Z. S., Duan, C. W., Zhao, H. Y., Tang, S. Q., Wen, Q., Zhang, Y. Y., Lv, M., Wang, Y., Xu, L. P., Zhang, X. H., Huang, X. J., & Kong, Y. (2022, Mar 29). Dysfunctional bone marrow endothelial progenitor cells are involved in patients with myelodysplastic syndromes. *J Transl Med, 20*(1), 144. https://doi.org/10.1186/s12967-022-03354-2

Xu, D. H., Zhu, Z., Wakefield, M. R., Xiao, H., Bai, Q., & Fang, Y. (2016, Apr 10). The role of IL-11 in immunity and cancer. *Cancer Lett, 373*(2), 156-163. https://doi.org/10.1016/j.canlet.2016.01.004

Xu, L. L., Warren, M. K., Rose, W. L., Gong, W., & Wang, J. M. (1996, Sep). Human recombinant monocyte chemotactic protein and other C-C chemokines bind and induce directional migration of dendritic cells in vitro. *J Leukoc Biol, 60*(3), 365-371. https://doi.org/10.1002/jlb.60.3.365

Xu, Y. X., Talati, B. R., Janakiraman, N., Chapman, R. A., & Gautam, S. C. (1999). Growth factors: Production of monocyte chemotactic protein-1 (MCP-1/JE) by bone marrow stromal cells: Effect on the migration and proliferation of hematopoietic progenitor cells. *Hematology, 4*(4), 345-356. https://doi.org/10.1080/10245332.1999.11746458

Yamashita, M., & Passegue, E. (2019, Sep 5). TNF-alpha coordinates hematopoietic stem cell survival and myeloid regeneration. *Cell Stem Cell, 25*(3), 357-372 e357. https://doi.org/10.1016/j.stem.2019.05.019

Youssef, M., Ahmed, H. Y., Zongo, A., Korin, A., Zhan, F., Hady, E., Umair, M., Shahid Riaz Rajoka, M., Xiong, Y., & Li, B. (2021, Oct 19). Probiotic supplements: Their strategies in the therapeutic and prophylactic of human life-threatening diseases. *Int J Mol Sci, 22*(20). https://doi.org/10.3390/ijms222011290

Yue, X., Wu, L., & Hu, W. (2015). The regulation of leukemia inhibitory factor. *Cancer Cell Microenviron, 2*(3). https://doi.org/10.14800/ccm.877

Zhang, Y., Wu, J., Qin, T., Xu, Z., Qu, S., Pan, L., Li, B., Wang, H., Zhang, P., Yan, X., Gong, J., Gao, Q., Gale, R. P., & Xiao, Z. (2022, Dec). Comparison of the revised 4th (2016) and 5th (2022) editions of the World Health Organization classification of myelodysplastic neoplasms. *Leukemia, 36*(12), 2875-2882. https://doi.org/10.1038/s41375-022-01718-7

Zhao, N., Stoffel, A., Wang, P. W., Eisenbart, J. D., Espinosa, R., 3rd, Larson, R. A., & Le Beau, M. M. (1997, Jun 24). Molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases to 1-1.5 Mb and preparation of a PAC-based physical map. *Proc Natl Acad Sci U S A, 94*(13), 6948-6953. https://doi.org/10.1073/pnas.94.13.6948

Zhou, B. O., Yu, H., Yue, R., Zhao, Z., Rios, J. J., Naveiras, O., & Morrison, S. J. (2017, Aug). Bone marrow adipocytes promote the regeneration of stem cells and haematopoiesis by secreting SCF. *Nat Cell Biol, 19*(8), 891-903. https://doi.org/10.1038/ncb3570

Appendix A: IRB Approval Letters

Office of Research Integrity

March 27, 2023

Lahari Kondeti
1675 6th Avenue, Apt. 2
Huntington, WV 25703

Dear Lahari:

This letter is in response to the submitted thesis abstract entitled *"Elucidating the Impact of Western Diet on Bone Marrow Cytokine Signatures: Understanding Poor Outcomes in Susceptibility to Infection and Progression of Cancer."* After assessing the abstract, it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocols #807 and #726. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract, you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director

Animal Resource Facility

DATE:	November 23, 2022
TO:	Melinda Varney, PhD
FROM:	Marshall University IACUC
IACUC #:	807
PROJECT TITLE:	[1975849-2] Impact of obesity on hematopoietic stem and progenitor cell response to influenza vaccination
SUBMISSION TYPE:	New Project
ACTION:	APPROVED
APPROVAL DATE:	November 23, 2022
EXPIRATION DATE:	November 22, 2025
REVIEW TYPE:	Full and Designated Member Review

Thank you for your submission of Revision materials for this research project. The Marshall University IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Full and Designated Member Review.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

Appendix B: Abbreviations

AML- Acute myeloid leukemia

BMI – Body mass index

BMP-2 – Bone morphogenic protein

CCL3 – Chemokine(C-C motif) ligand 3

CSF – Colony stimulating factor

CXCL12- C-X-C Motif chemokine ligand 12

DKO- Double knock out

GATA – Erythroid transcription factor

G-CSF – Granulocyte colony stimulating factor

Gfi 1- Growth factor independent transcriptional repressor

HGF- Hepatocyte growth factor

HSC- Hematopoietic stem cells

HSPC – Hematopoietic stem and progenitor cells

IAV- Influenza A virus

IKKB- Inhibitor of k kinase

IL-6 – Interleukin 6

JNKs- cJun-amino terminal kinases

K2EDTA- Dipotassium ethylenediamine tetra acetic acid

LIK – Leukemia inhibitory factor

LSK- Lineage- sca1+ckit+

MCP-1/3 – Macrophage chemoattractant protein-1/3

M-CSF – Macrophage colony stimulating factor

MDS- Myelodysplastic syndrome

MPP- Multipotent progenitor

mRNA- Messenger ribonucleic acid

MSCs- Mesenchymal stem cells

NK cells- Natural killer cells

PAI1- Plasminogen activator inhibitor1

PBS – Phosphate buffered saline

PF4- Platelet factor 4

PGE2 – Prostaglandin E2

PKR- Protein kinase R

PPARγ – Peroxisome proliferated activated receptor gamma

RANKL – Receptor activator of nuclear factor kappa-B ligand

SCF- stromal cell factor

SDF-1 – Stromal cell derived factor

TGF-β1 – Transforming growth factor

TLRs- Toll like receptors

TNF-α – Tumor necrosis factor alpha

Appendix C: C Series Cytokine Array Maps (Raybiotech)

C3 Array

Each antibody is spotted in duplicate vertically

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1 2	POS	POS	NEG	NEG	BLANK	Axl	BLC (CXCL13)	CD30 Ligand (TNFSF8)	CD30 (TNFRSF8)	CD40 (TNFRSF5)	CRG-2	CTACK (CCL27	CXCL16	Eotaxin-1 (CCL11)
3 4	Eotaxin-2 (CCL24)	Fas Ligand (TNFSF6)	Fractalkine (CX3CL1)	GCSF	GM-CSF	IFN-gamma	IGFBP-3	IGFBP-5	IGFBP-6	IL-1 alpha (IL-1 F1)	IL-1 beta (IL-1 F2)	IL-2	IL-3	IL-3 R beta
5 6	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40/p70	IL-12 p70	IL-13	IL-17A	KC (CXCL1)	Leptin R	Leptin	LIX	L-Selectin (CD62L)
7 8	Ltn (XCL1)	MCP-1 (CCL2)	MCP-5	M-CSF	MIG (CXCL9)	MIP-1 alpha (CCL3)	MIP-1 gamma	MIP-2	MIP-3 beta (CCL19)	MIP-3 alpha (CCL20)	PF-4 (CXCL4)	P-Selectin	RANTES (CCL5)	SCF
9 10	SDF-1 alpha	TARC (CCL17)	I-309 (TCA-3/CCL1)	TECK (CCL25)	TIMP-1	TNF alpha	TNF RI (TNFRSF1A)	TNF RII (TNFRSF1B)	TPO	VCAM-1 (CD106)	VEGF-A	BLANK	BLANK	POS

C4 Array

Each antibody is spotted in duplicate vertically

	A	B	C	D	E	F	G	H	I	J	K	L
1 2	POS	POS	NEG	NEG	BLANK	bFGF	CD26 (DPPIV)	Dtk	E-Selectin	Fc gamma RIIB	Flt-3 Ligand	GITR (TNFRSF18)
3 4	HGFR	ICAM-1 (CD54)	IGFBP-2	IGF-1	IGF-2	IL-15	IL-17 RB	IL-7	I-TAC (CXCL11)	Lungkine (CXCL15)	MDC (CCL22)	MMP-2
5 6	MMP-3	OPN (SPP1)	OPG (TNFRSF11B)	Pro-MMP-9	Resistin	Shh-N	TCK-1 (CXCL7)	TIMP-2	TRANCE (TNFSF11)	TROY (TNFRSF19)	TSLP	VEGFR1
7 8	VEGFR2	VEGFR3	VEGF-D	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	POS

C5 Array

Each antibody is spotted in duplicate vertically

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1 2	POS	POS	NEG	NEG	4-1BB (TNFRSF9)	6Ckine (CCL21)	ACE	ALK-1	AR	CT-1	CD27 (TNFRSF7)	CD27 Ligand (TNFSF7)	CD36 (SR-B3)	CD40 Ligand (TNFSF5)
3 4	Chordin	CTLA-4 (CD152)	Decorin	DKK-1	E-Cadherin	EGF	Endoglin (CD105)	Epigen	Epiregulin	Galectin-1	Gas 1	Gas 6	GITR Ligand (TNFSF18)	Granzyme B
5 6	HAI-1	HGF	IL-1 R4 (ST2)	IL-11	IL-17B	IL-17E (IL-25)	IL-17F	IL-1 RA (IL-1 F3)	IL-2 R alpha	IL-20	IL-21	IL-28A	IL-6 R	JAM-A (CD321)
7 8	MAdCAM-1	MFG-E8	Neprilysin	Pentraxin-3 (TSG-14)	Prolactin	RAGE	TACI (TNFRSF13B)	TREM-1	TWEAK (TNFSF12)	TWEAK R (TNFRSF12)	NEG	NEG	NEG	POS