The Impact of Iron Deficiency Anemia on Natural Killer (NK) Cell Activity, Growth, Production, Cytotoxicity, and Neuroimmune Interactions.

A CYSF research paper

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The primary objective of this literature review is to assess all correlations between iron deficiency anemia and natural killer cell activity, growth, production, cytotoxicity, and neuroimmune interactions in order to establish possible therapeutic and medical interventions for patients with iron deficiency anemia. This paper will refer to iron deficiency anemia as IDA, while natural killer cells will always be referred to as NK cells. An anemia is a condition where an individual's body produces a lower than normal amount of healthy blood cells and/or hemoglobin, which leads to an oxygen deficient blood system that does not provide the required amount of oxygen to the body (*NIH 2022).* An iron deficiency anemia, or IDA is an anemia that occurs due to a lack of iron in the blood system, which is caused by a plethora of circumstances such as but not limited to, insufficient iron intake, blood loss and impaired iron absorption (*Mayo clinic 2022*). Natural killer cells are a primary component of the innate immune system, the innate immune system is the body's first line of defense against all potential threats such as viruses, bacteria and cancerous cells. Non-specific and rapid defence is how the innate system operates so that it may provide speedy initial defence against all types of threats, the innate immune system provides this defence through protection offered by immune system cells, proteins, and skin and mucous membranes.The natural killer cells are the third major part of the innate immune system. The NK cells main job is to identify cells that have been infected by a virus, as well as abnormal cells that may turn into (or have turned into) tumor cells. To do this, they search for cells with an abnormal or unfamiliar surface, and then destroy the cell surface using substances called cytotoxins (*NIH 2023*).

Fundamentally, NK cells are a type of innate immune system white blood cells that destroy infected, stressed and diseased cells such as cancer cells or virus/bacteria infected cells through spontaneous cytolytic activity. They comprise 5-15% of human peripheral blood cells and are a subset of group 1 innate lymphoid cells (ILCs), originating from hematopoietic stem cells, producing IFN-γ and relying on the transcription factor Tbet. Being a part of group 1 innate lymphoid cells, means that they are a type of lymphocyte like b or t cells. Despite originating from the same lymphoid progenitor as acquired immunity cells (T and B cells), NK cells function differently. Nk cells have the ability to destroy harmful cells in the early stages, preventing viruses and cancer cells from spreading. Due to the NK cell being a part of the innate system, they are the body's first line of defence and will destroy threats before the body is impacted at all. This means that NK cells will kill and destroy threats without its human host ever noticing a thing or being any wiser. NK cells are called “natural” killers because they can destroy potential threats without prior exposure to a particular pathogen, unlike other lymphocytes such as cytotoxic T cells that need previous exposure to a pathogen before they can eliminate the threat.The purpose of an NK cell is to quickly destroy once healthy cells that now pose a threat to the human body, such as cancerous or infected cells. An NK cell will patrol the body in search of cell markers that indicate whether or not a cell is healthy and a part of the human body (self) or if the cell is infected or diseased (not self). If an NK cell decides that they have found an infected, diseased or cancerous cell, the NK cell will release cytotoxins such as perforin or granzymes that will kill a cell target.Activated NK cells release cytokines that tell other white blood cells to help rid your body of the threat. Natural killer cells are not designed to attack cells it recognizes to be healthy and a part of the body, an NK cell distinguishes self from invader using cell markers. The MHC-1 cell marker is the most common cell marker an NK cell recognizes as belonging or “self.” The MHC-1 on the target cell attaches (binds) to an NK cell’s inhibitory receptor which turns the NK cell’s killing function off. An NK cell will always attack damaged cells, and will never attack an entirely new entity such as a bacteria. However, Nk cells do impact bacterias, as they use cytokines to report the bacterias to other immune cells. Moreover, cancer cells and infected cells will also release activating signals that cause NK cells to attack, and NK cells will also attack cells that have downgraded MHC-1 which can be caused by a plethora of things, of which most common is a viral infection . NK cells develop from common lymphoid progenitor cells (CLPs) that primarily reside in the bone marrow,liver and thymus. Maturation of NK cells involves stages marked by specific receptor expression (e.g., CD117, CD122, NK1.1, NKp46, CD49b, CD11b, CD27). As NK cells continue to develop, they may stay in an individual's bone marrow or move to other tissue and organs in the lymphatic system, such as the lymph nodes, spleen, tonsils and thymus. Once NK cells have matured, the body will release them into the bloodstream, however NK cells also

exist in lymph tissues and associated organs such as the lungs and liver. Interestingly, about 5 to 10 percent of the lymphocytes in a body's blood are NK cells and at any time an adult body will have more than 2 billion nk cells. Yet Nk cells have a short lifespan, living only 2 weeks (*Cleveland clinic 2025*). Moreover, Human NK cells are diverse. CD56bright NK cells produce cytokines but lack spontaneous killing ability, while CD56dim NK cells are primarily cytotoxic. Consequently, Key differences to note concerning Nk cells and other immune cells, are that Nk cells do not have the memory response of T or B cells, They don't require antigen presentation for target cell identification and killing, and that they release interferon gamma, similar to T helper 1 cells, activating macrophages. Unlike other innate immune cells, NK cells can also exhibit immunological memory, either through antigen-dependent or antigen-independent mechanisms. This is relevant in viral infections and cancer responses.

Friendly Neighborhood Immunologist. (2021, September 9). *Natural Killer cells | Top 5 ways Natural Killer cells work* [Video]. YouTube. https://www.youtube.com/watch?v=aBDhnZrxAaY

Drbeen Medical Lectures. (2022, November 10). *Summary - Natural killer (NK) cells* [Video]. YouTube. https://www.youtube.com/watch?v=arz-sYRgLnk

Professor Dave Explains. (2023, December 20). *Natural killer cells: the tumor killers* [Video]. YouTube. https://www.youtube.com/watch?v=iATp8DO3RA8

Cell activity

In essence, basic biology states that cell activity refers to all the functions and procedures a cell can do and is done inside a cell. According to *Current opinion in microbiology , 2010* a basic cell activity is a proper response and adaptation to diverse extracellular challenges. A response or adaptation to an extracellular challenge refers to how a cell reacts and adjusts to changes in its environment, which can be various external factors like temperature, pH, nutrient levels, or the presence of signaling molecules. In a nutshell any garden variety cell will respond and adapt to extracellular challenges through signal reception, signal transduction, feedback mechanisms and cellular responses such as metabolism, gene expression, movement, and cell cycle regulation. NK cell activity refers to all the processes, procedures, and functions done by an nk cell. Since the purpose of an NK cell is to hunt and kill threats and invaders to the human body, Most often NK cell activity specifically refers to how NK cells recognize, respond, and eliminate abnormal or infected cells. Nk cell activity is often measured through cytokine production, cytotoxin production, receptor expression, reactivity to MHC1, release of perforins and granzymes, changes in NK cell metabolism, and NK cell activation and degranulation. Furthermore NK cell activity heavily depends on activating and inhibitory receptors. Inhibitory receptors (e.g., NKG2A, Ly49A) bind MHC class I, promote self-tolerance. Loss of MHC class I on stressed cells, combined with upregulation of activating receptor ligands, triggers NK cell activation. Consequently, Cytotoxicity involves immunological synapse formation, degranulation (perforin, granzyme release), and death receptor-mediated apoptosis. Activated NK cells release various cytokines (IFN-γ, TNF-α, etc.) and chemokines. Uniquely, NK cells can exhibit immunological memory, either antigen-dependent or -independent. NK cell activity is also enhanced by signals from other immune cells, such as Macrophages which release interleukin-12, and dendritic cells which release interferon alpha and beta which all activate NK cells. NK cells also utilize antibody-dependent cellular cytotoxicity (ADCC) via IgG receptors. Other mechanisms of NK cell mediated cell death that are not perforin and granzymes include Fas ligand,TNF alpha and interferon gamma (IFN-Y). Moreover, NK cells heavily rely on transaction factor Tbet in order to properly function. Supplementary focus will be put towards NK cell recognition of threats. This part of the paper will attempt to cover all of the aforementioned ways NK cells recognize, respond and eliminate abnormal or infected cells in relation to IDA , in order to establish a connection and discuss possible medical and therapeutic interventions for patients with IDA.

Friendly Neighborhood Immunologist. (2021, September 9). *Natural Killer cells | Top 5 ways Natural Killer cells work* [Video]. YouTube. https://www.youtube.com/watch?v=aBDhnZrxAaY

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According to *Hallquist, N. A. et Sherman, A. R 1989* Spleen natural killer (NK) cell activity and protein synthesis are decreased in iron-deficient rats. IFN production or ability to stimulate NK cells is decreased by iron deficiency, but syngeneic B cells compensate for the decreased activity. This is significant because IFN plays a role in decreasing NK cell activity during iron deficiency. Researchers in this study took weanling male rats (n=9/group) and fed them ad libitum 5 or 37 mg iron/kg diet for eight weeks. Rats who were pair fed (n=6) were fed a control diet which was the same as iron-deficient rats. Afterwards researchers measured spleen NK cell activity by Cr-51 release from labeled Yac-1 cells, directly after NK cells were activated by macrophage-produced IFN. In order to produce IFN, Macrophages were stimulated in vitro with poly inosinic:cytidylic acid. Interferons (IFN) are signaling proteins that help to activate NK cells. Production of IFN by macrophages are low in iron-deficient rats which causes impaired NK cell activity. Furthermore, hemoglobin and hematocrit levels were lower in iron-deficient rats than in other rats, which is significant because both are necessary for oxygen transport. Oxygen transport is crucial for NK cells because they rely on it for energy production through oxidative phosphorylation (OXPHOS), which is essential for NK cell survival, basic function and their cytotoxic and cytokine-producing functions. NK cell cytotoxicity after incubation with macrophage-produced IFN is decreased in iron-deficient rats (13.4±1.5%) compared to control rats (19.0±1.9%), while increased NK activity was found in higher amounts in pair-fed rats (25.5±1.9%) compared to control and iron deficient rats. Proving that IDA affects NK cell activity in a negative manner through negatively affecting protein synthesis, IFN production, cytokine producing functions and cytotoxic mechanisms.

Furthermore, a literature review completed by (*Beisel, W. 1982*) states that lymphocyte count, In vitro lymphocyte transformation,antibody production, dermal hypersensitivity, chemotaxis, Phagocytosis, bactericidal activity, and metabolic c3 are all impacted by IDA, however through extensive research no correlation is found between IDA and NK cell towards cell activity. However, analysis of multiple sources through literature review comes to the following data conclusion. Increased susceptibility to infection due to IDA via lymphoid tissues and reduced lymphocyte and NK cell counts, may lead to impaired In vitro lymphocyte transformation. While normal or impaired antibody production may have decreased delayed dermal hypersensitivity and may have increased or impaired chemotaxis, or phagocytosis impaired bactericidal activity. Metabolic c3 may increase. However no exact correlation was found between IDA and NK cell activity. Specifics for NK cell activity are denied in the literature review and so are the correlations between cell activity and IDA. Lymphocyte transformation however, is defined as the in vitro process where lymphocytes are stimulated to proliferate and change in size and appearance in response to an external threat which is exactly the definition of NK cell function and activity. However, lymphocytes are a cell subtype that include an array of cells, of which are T cells, B cells and NK cells. Consequently, due to the fact that lymphocyte transformation includes multiple cells, a true conclusion cannot be made. However, if one were to assume that lymphocyte transformation refers to NK transformation and results are not altered by other cells that are a part of the lymphocyte subtype. Then the conclusion could be made that since lymphocyte transformation is affected by IDA, NK cell transformation must also be affected by IDA. Specifics on positivity or negativity towards the connection between NK cell transformation and IDA are not given in the study. However the overall conclusion of this paper is that there is a minimal connection between IDA and NK cell activity and that the overall effect is net zero.

Another study done by (*Rosch, L. M et al 1987*) titled Iron deficiency impairs protein synthesis in immune tissues of rat pups, which was published in the Journal of Nutrition claims that protein synthesis in the liver and

thymus was not changed by moderate iron deficiency. Moderate iron deficiency did however, impact protein synthesis in the spleen. In the spleen, liver and thymus, protein synthesis in severely iron-deficient pups was less than half that of iron-sufficient pups.The study measured RNA, DNA and in vitro protein synthesis in rats that were fed diets containing multiple levels of iron. The first severe anemia diet consisted of 6 mg iron/kg , the second moderate anemia diet had 11 mg iron/kg and the third iron sufficient diet had 250 mg iron/kg throughout gestation and lactation. Each day of lactation researchers altered the experiment landscape. On day 2 of lactation, litters were adjusted to contain six pups. On day 12 of lactation, two pups from each litter were immunized with sheep red blood cells (SRBC) and on day 17, tissues were removed for the determination of protein synthesis and evaluation of RNA and DNA contents. In the moderately iron-deficient pups, protein synthesis was significantly lower (30%) in the spleen than that in the iron-sufficient pups. Protein synthesis in the spleen of the moderately iron-deficient group was higher after immunization with SRBC than in iron-sufficient controls, whereas the severely iron-deficient pups failed to respond. Impaired protein synthesis may be the mechanism responsible for compromised ability to produce antibodies in iron deficiency. These findings are significant towards IDA and NK cell activity because protein synthesis is necessary for NK cell function. Protein synthesis is crucial for NK cell activity because it is crucial for all basic cell function. Moreover protein synthesis also underpins the production of proteins essential for all NK cell function, including receptors, effector molecules (like perforin and granzymes), and signaling molecules, all of which are vital for NK cell recognition, activation, and target cell destruction- of which NK cells cannot activate or function without.

Consequently, another study titled “Impaired natural killer cell activity in Iron-Deficient rat pups” published in the journal of nutrition written by (*Sherman et al 1987*) concluded that IDA significantly impaired spleen NK cell activity when measured by two different effector:target ratios and assay time periods. Researchers studied NK cell activity through experimentation on iron-deficient rat pups. Pregnant dams were fed diets containing 6, 10 or 250 ppm Fe ad libitum from d 1 of gestation through d 21 of lactation. After two days post parturition, litters were adjusted to seven pups each, and on day 17 the pups were injected intraperitoneally with 5 × 105 plaque-forming units of vaccinia virus. Following a 4-d incubation period, spleens were removed and the cell suspensions were combined with YAC-1 target cells to measure cytolysis in a 4- and 16-h chromium release assay. Findings show that hematocrit levels of severe (6 ppm) and moderately (10 ppm) iron-deficient rat pups were significantly lower than that of controls. Similarly, body weight and spleen weights were significantly lower in iron deficient pups than in control pups. Further evidence was not provided by the study due to access issues, however conclusions towards NK cell activity were provided. The study concluded that IDA consistently and significantly affected the activation and functioning of NK cells in the spleen.

Moreover, according to *Chegni, H., & Taheri, M. S. 2024* Iron deficiency can affect the function of natural killer cells (NK cells) because the activity of these cells increases the expression of transferrin receptors. IDA can also reduce the release of interferon gamma in viral infections. The role of iron on these innate immune cells (NK cells) is clear, especially in tumor cells. Knowledge of relations between IDA and tumors can be used to increase the antitumor activity of natural killer cells by chelating iron, although using iron chelators such as deferoxamine (DFO) is limited due to side effects on other cells. This literature review was completed through analysis of previous work and sources were obtained from relevant studies published from January 1980 to January 2024 and indexed in PubMed, Google Scholar, Ovid and Scopus. The keywords used in the search were iron deficiency anemia, innate and adaptive immune system, cellular and humoral immunity. Evidence towards NK cell activity and IDA being interconnected lies in the following. In the tumor environment (TME), natural killer cells play a role in tumor destruction by producing cytotoxic cytokines Through increasing the expression MHC1 due to the presence of inhibitory receptors, the activity of NK cells decreases, yet on the contrary, when the amount of iron and ferritin heavy chain (FTH) decreases, the level of MHC1 expression also reduces and the vulnerability of cancer cells to NK cells increases. Therefore IDA results in decrease of MHC1

expression which leads to reduced natural killer function, cytotoxicity and vulnerability to cancer thus reducing NK cell activity.

A total of 200 children participated in a study for (*Rahmani, S., & Demmouche, A. 2015*) concerning Iron deficiency anemia in children and alterations of the immune system. 101 of these children who participated in the study group were diagnosed to have iron deficiency anemia which was related to nutritional deficiency. While 99 healthy children at the same ages participated in the control group. The complete blood count (automatic cell analyzer 600), serum iron (spectrophotometry) and ferritin (RIA) in all children were measured. Moreover, a measure of 1 ml of blood sample with EDTA-containing tubes was taken by venipuncture from each patient for complete blood count, including differential cell counts, hemoglobin, hematocrit, serum IgG, IgM, IgA, and ferritin levels. Whole blood samples were also collected. Serum immunoglobulins were measured by using commercially prepared antisera to IgG, IgA, IgM. and radial immunodiffusion methods. The data collected by the study lists is as follows. GALAN and Al reported a reduction in the production of interleukin-2 by lymphocytes activated in iron-deficient patients. The release of interleukin-2 is fundamental to communication between lymphocyte subpopulations and natural killer cells, but it doesn't seem to be the only cytokine which is modified by the iron status [29-31]. The percentage of lymphocytes was 43,681 ± 17,936% in children with IDA and 38,199 ± 16,699% in the control group ($P<0.026$). No correlation was found between lymphocytes and hemoglobin ($r=-0.18$).Reported immune defects in iron deficiency include decreased cell-mediated immunity, mitogen responsiveness and natural-killer cell activity. Conclusions for the study stated that reported immune defects in iron deficiency include decreased cell-mediated immunity, mitogen responsiveness and natural-killer cell activity. There is also reported reduction in production of interleukin-2 cytokines that lead to decreased communication between NK cell and lymphocyte subpopulations, which lead to disruption in immune responses, potentially leading to increased susceptibility to infections and autoimmune disorders. This evidence is significant because of the alterations found in cytokine levels of children with IDA. Reductions in populations of cytokines such as interleukin-2 detrimentally affect the communication between lymphocyte subpopulations and natural killer cells. Moreover cytokines such as interleukin-2 are extremely necessary for basic NK cell activation and recognition of threats as well as cytotoxic killing.
The reduction of iron and FTH may influence the expression of MHC class I molecules leading to NK cells activation because intracellular ferritin levels affect the abundance of MHC class I antigens on cell surface. Moreover, data suggest that labile iron acts directly on IFN-γ signaling by obstructing STAT1 activation, while intracellular ferritin abundance does not, only impinging MHC class I expression on the cell surface. Furthermore the iron/ferritin down modulation leads to a highly NK cell susceptible immune phenotype with low surface levels of MHC class I inhibitory molecules and increased amount of membrane associated activating DNAM-I ligands. These findings were observed by (*Sottile, R. et al 2019)* through a study titled Iron and Ferritin modulate MHC Class I expression and NK cell recognition, published by Frontiers in Immunology. Researchers in this study took MM07m (supraclavicular lymph node metastasis) and MM07m shFTH (FTH-silenced) cells from human and rat spleens and cultured them in RPMI 1640 (Life Technologies) with 10% FBS, 10 units/ml penicillin, and 10 mg/ml streptomycin. Researchers also took MCF-7 and MCF-7 shFTH cells and cultured them in Dulbecco's modified Eagle's medium (Life Technologies) with the same supplements. Cells were grown at 37°C in a 5% CO2 atmosphere. NCOA4 WT and KO mice (C57BL6 background) were maintained under specific pathogen-free conditions. NCOA4 WT and KO in C57BL6 genetic background mice were maintained under specific pathogen-free conditions and splenocytes were isolated by mechanical disruption, treated with RBC lysis buffer, and washed. Splenocytes were then processed for RNA or protein extraction. Multiple tests, including NK Cell Generation Assay, Cytotoxicity Assay, FACS analysis, LIP measurements, Calcein Test, Protein Studies, and RNA Isolation for q-PCR, were performed. Significance of this data is due to the increased expression of MHC class one molecules, because they are necessary for the recognition and activation of NK cells. Moreover the increases in interferon gamma is also significant because interferon gamma is essential to NK cell activity, due to

Interferon-gamma (IFN-γ) being crucial for NK cell activity because it enhances cytotoxic functions, promotes accumulation, activation, and contributes to the development of an effective antitumor and antiviral response.

A scoping based literature review, focused on examining the impact of iron on Cancer-Related Immune Functions in Oncology (*Badran et al 2024*) came to the conclusion that Iron plays a critical role in maintaining the cytotoxic function of NK cells. When iron levels are insufficient, the cytotoxic activity of NK cells is significantly reduced. Moreover, Iron is essential for NK cells' proper activation and function. Without adequate iron, NK cells struggle to produce the necessary cytotoxic molecules, which affect their ability to eliminate tumor cells effectively. Reduction in NK cell activity negatively affects cancer due to the fact that NK cells are essential for controlling tumor growth. Evidence for this lies in the fact that IDA impairs the production of IFN-γ, a key cytokine produced by NK cells that stimulates other immune cells and enhances tumor cell destruction .The reduced output of IFN-γ weakens the overall immune response, further diminishing the ability of NK cells to control tumor growth and negatively affecting NK cell activity and function. Interferon gamma is also a key cytokine involved in communication between lymphocytes, decrease in this cytokine has detrimental impacts of cell activity related to communication.

An experimental article titled "Analysis of Natural Killer cell functions in patients with hereditary hemochromatosis." was published in 2020 by (*Bönnemann et al 2020*). This article concluded that no major changes in the phenotype, cytokine or the cytotoxic function of NK cells in HH patients were found. HH represents hemochromatosis which is a disorder where extreme iron builds up. Analysing NK cell activity in relation to HH could reveal conclusions for NK cell activity in relation to IDA. The team in this study examined immune cell phenotype and function in 21 HH patients compared to 21 healthy controls with a focus on Natural Killer (NK) cells. They observed increased basal and stimulated production of pro-inflammatory cytokines such as IL-1β or IL-18 in HH patients compared to healthy controls. The data they received indicates a general decrease in the total number of granulocytes in HH patients (2774 ± 958 per µl versus 3457 ± 1122 per µl in healthy controls).However they did not find any other significant changes.Demonstrating that NK cells of HH patients are not significantly affected and that the patients' treatment by regular phlebotomy is sufficient to avoid systemic iron overload and its consequences to the immune system. Net zero effects proven to have been achieved by hemochromatosis in relation to NK cell activity establish a net zero conclusion for the relation between IDA and NK cell activity, if it is assumed that HH and IDA have a parallel relation with each other with equal and adverse implications.

In 2024 a cohort of PWO's, people with obesity who were confirmed to either have or not have IDA were tested on multiple NK cell factors and cytotoxicity. The data the researchers collected demonstrates that in response to cytokine stimulation, healthy human NK cells utilize iron to support their metabolic activity and cytokine responses. The study also demonstrates alterations in NK cell metabolism, mitochondrial fitness and cytokine production. Furthermore, upon stratification into PWO with normal iron status versus low iron status,the observed obesity-related defects in NK cell metabolism, mitochondrial fitness and cytokine production are concentrated in the PWO with low-iron status. Leading to the conclusion of the fact that obesity related IDA is associated with significant defects in the functionality of human NK cells, especially in the periphery. Dysregulated cellular metabolism has been demonstrated to be a major mechanistic driver of the reported defects. However, how obesity itself links to defective NK cell metabolism and functionality remains unclear. The significance of this study was that it was able to prove that NK cells that activate and function properly need an adequate amount of iron to support their metabolic activity, and cytokine responses. Moreover it was proved that PWO patients with IDA had defects in NK cell metabolism, mitochondrial fitness and cytokine production. All of these activities that NK cells complete are very important. For example cytokine production is how NK cells communicate with other cells and establish immune response, cytokines are also necessary for cytotoxic functions that NK cells use to kill threats to the human body. While mitochondrial fitness refers to the health of the mitochondria, the powerhouse of

the cell of which without the NK cell cannot complete any of its functions, activities or be activated. Moreover, even though NK cell metabolism is most necessary for NK cell growth as specified in this paper, it is also necessary for normal day to day function for an NK cell as NK cells cannot activate or function without the energy and molecular building blocks processes that support both growth and functionality. Therefore, decrease in metabolic activity, mitochondrial fitness and cytokine production of NK cells in relation to IDA prove that IDA has a negative impact on NK cell activity.

Furthermore, a study titled focused on analysing alterations of NK cells using proteomics analysis in patients with severe aplastic anemia, found that many DEPs involve dysfunction of NK cells, which provides potential targets for deeper research of inadequate immunomodulation, Moreover, they also found that there is positive correlation between SAA patients and dysfunction of NK cells (*Liu. H 2020*). SAA is a disease named severe aplastic anemia that occurs due to dysfunction in the bone marrow that causes a lack of creation of blood cells. SAA is often correlated with hemochromatosis or the excess buildup of iron in the body, therefore changes in NK cell activity with SAA should correlate to changes in NK cell activity in relation to iron buildup and deficiency. In the study, researchers took peripheral blood NK cells from both SAA patients and normal controls, the blood NK cells were then sorted and total proteins were extracted. After sorting and extraction, mass spectrometry was performed to screen differentially expressed proteins (DEPs). Differentially expressed proteins are proteins whose abundance or activity varies significantly between different conditions or groups, as determined through statistical analysis of proteomic data. DEP's are meant to ring the alarm bell for dysfunctional cells that are not activated well. Significant differences in the expression levels of 93 proteins were observed in NK cells of SAA patients compared with normal controls. Among them, 48 were upregulated proteins, including histone H1.2, histone H1.3, heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1), and interferon regulatory factor 1 (IRF-1), and 45 were downregulated proteins, including actin-related complex (ARP2/3), histone H3, histone H4, phosphoglycerate kinase 1 (PGK1), talin-1. Gene Ontology (GO) function indicated that the DEPs most involved were vesicle-mediated transport, innate immune response, and DNA binding. Succinctly, this indicates that there is a positive correlation with IDA and NK cell activity and DEP'S, due to the fact that excess iron in SAA resulted in a negative correlation with NK cell activity- where excess iron buildup led to decrease in NK cell activity. Increase in DEP's correlates with decrease in cell activity because an increase in proteins that do not function normally should and will correlate with cells that are not activating or functioning in a proper manner.

Researchers publishing a paper in anticancer research journal (*Jiang, X., et Elliott, R. L 2017*) manipulated intracellular iron levels of the human MCF-7 and MDA-MB-231 breast cancer cell lines, and measured cytolysis of breast cancer cells by the natural killer cell line NK-92MI, nitric oxide (NO) production, tumor necrosis factor alpha (TNFα) production and gene expression of ferritin heavy chain (FTH1). The aim of these methods and procedures was to assess decreased iron in cancer cells and how their microenvironment improves cytolysis of breast cancer cells by natural killer cells. Researchers found that NK-92MI increased synthesis and release of NO and TNFα into the medium during co-culturing of NK-92MI cells with MCF-7 or MDA-MB-231 cells. Addition of iron inhibited the cytolysis of the breast cancer cell lines. The iron chelator deferoxamine (DFOM) increased NK-92MI cytolysis to MCF-7 or MDA-MB-231 cells. Iron reversed cytotoxicity to breast cancer cells induced by NO, released from S-nitroso-N-acetyl-penicillamine (NO donor). Real time quantitative polymerase chain reaction showed that iron up-regulated the expression of FTH1 and iron chelator DFOM reduced FTH1 expression of MCF-7 and MDA-MB-231 cells. These findings led to the conclusion that increased iron in cancer cells and their microenvironment protects cancer cells from natural killer cell cytolysis by antagonizing NO- and TNFα-associated cytotoxicity and by up-regulation of ferritin expression in breast cancer cells. Conversely, a decrease in iron concentration caused by DFOM improves natural killer cytolysis of tumor cells. Proving that IDA leads to improved natural killer cytolysis of tumor cells, while increased iron leads to decrease in NK cytolysis of tumor cells.

Furthermore, in a 1988 article titled “The effect of iron, iron‐binding proteins and iron‐overload on human natural killer cell activity” , researchers assessed the in vitro natural killer (NK) activity of peripheral blood lymphocytes (PBL) in 13 patients with genetic haemochromatosis (HC) and 27 normal subjects, using a 51Cr-release cytotoxicity assay against the target K-562 leukaemia cell line (*Chapman, D. E et al 1988*). Hemochromatosis is the excess buildup of iron in the blood system, and relations between hemochromatosis and NK cell activity will shed light on IDA’s effect on NK cell activity. Mean NK function did not differ between the two groups of hemochromatosis patients and non patients. According to the article, this conclusion differs from the reported deficit in NK activity in other diseases in which increased iron stores may occur, including alcoholic cirrhosis and β-thalassaemia major.The results of this study suggest that peripheral blood NK function is not compromised in haemochromatosis. Since NK cell activity is not compromised nor helped in hemochromatosis, it can be assumed there was no relation between IDA and NK cell activity found in this study.

According to a scoping based literature review titled iron metabolism and immune regulation (*Ni, S. et al 2020*), in innate immunity, iron regulates macrophage polarizations, neutrophils recruitment, and NK cells activity. Moreover, according to the article iron also plays a pivotal role not only in the development and proliferation but also in the activation and function of NK cells when virus infection occurs. The findings in this article that pertain to NK cell activity include activation and function of NK cells that were found to be affected by iron. According to the article, iron is essential for the activation of NK cells, because iron inhibits differentiation and activation of Th1, Th2, Th17 and Treg cells, but it also promotes CTL differentiation. Additionally IDA results in a loss of function in NK cells due to negative effects on cytokines such as interferon (IFN)-γ, and tumor necrosis factor (TNF) interleukin-2, IL-12, IL-15 and IL-18. Activated NK cells increase expression of transferrin receptor (CD71) while IDA results in decrease of expression in CD71. Moreover an increased absorption of iron was found followed by NK cells activation, which means a decreased iron absorption would lead to a lack of activation of NK cells. Furthermore, subtypes of iron-absorption NK cells are CD27+ CD11b+ NK cells. Moreover, systematic low iron levels influenced by hepcidin resulted in the suppression of NK cell activation and production of IFN-γ. Sufficient serum iron is critical to the metabolism of NK cells and their activity against virus infection. Proving that iron is critical to the activation of NK cells.

Moreover iron deficiency profoundly impaires NK cell antiviral functions, leading to increased viral loads. Iron is very important for essential cellular activities including mitochondrial function, DNA repair and synthesis, and epigenetic regulation and sensing of hypoxia.The antiviral functions of NK cells strongly depend on sufficient levels of iron. Additionally, NK cells have an increased demand for iron when responding to acute FV infection and that experimental reduction of serum iron levels inhibits NK cell activation and their IFNγ production (*Littwitz-Salomon, E. et al 2021*). In this study C57BL/6 mice were infected with 40,000 Spleen Focus-Forming Units (SFFU) of FV and the viral loads in the bone marrow and spleens were monitored over the course of 28 days. The bone marrow and spleen were analysed due to high viral replication in these organs25. Since FV preferentially infects erythroblasts, monocytes and macrophages but all dividing cells in the spleen and bone marrow can be targets for infection, iron and Nk cells were tested after the infection. Findings suggest that there was an increase in the uptake of transferrin into NK cells in FV-infected mice compared to NK cells from naive mice. Increased transferrin uptake and CD71 expression were observed in both NK cell populations from the bone marrow and the spleen following infection, suggesting that responding NK cells have an increased demand for iron.CD71 expression and transferrin uptake are mainly represented by by CD27+CD11b+ NK cells. Analysis of NK cell activation revealed a significant decrease of CD69+ NK cells in animals with reduced levels of serum iron upon acute FV infection in the spleen and bone marrow. There was also a dramatic decrease of cMyc+ NK cells in mice with low serum iron upon FV infection. NK cells isolated from infected mice with low serum iron levels showed significantly less killing of FBL-3 target cells than NK cells isolated from infected mice with normal serum iron levels. This data suggests that iron is crucial for essential NK cellular activities such as mitochondrial function, DNA repair and synthesis, epigenetic regulation, sensing of hypoxia, cell activation,

cytotoxicity, cytokine production and antiviral functions. These key aspects of NK cell activities depend on iron, which means that severe IDA will detrimentally affect these aspects.

Iron Deficiency Anemia (IDA) is a universal health problem and a risk factor for the development of cancer. IDA changes the microenvironment of the human body by affecting both the biological and immunological systems. It increases DNA damage and genomic instability by different mechanisms. Due to IDA'S effects on the innate immune system and cytotoxicity it can be concluded that IDA leads to the deterioration of NK cell cytotoxicity that leads to negative effects on the bodys cancer detection and treatment (*Zohora, F. 2018*). The paper found that IDA disturbs multicellular signaling pathways involved in cell survival and helps in tumor angiogenesis. Moreover, IDA is also responsible for the functional deterioration of innate and adaptive immune systems that lead to immunological dysfunctions against invading pathogens. Cytotoxicity is an important part of cell activity and its deterioration due to IDA is an important factor for NK cell activities relation to IDA, however the most important piece of data from this study regarding cell activity is the the negative effects of IDA on the multicellular signaling pathways of cytokines that are involved in not only the survival of NK cells, but also tumor angiogenesis. Dysfunction in these signaling pathways prove that IDA impacts NK cell activation in a detrimental manner.

Consequently another study took twenty-two trained women runners (VO2peak 48.1 + 1.2 ml × kg-1 × min-1) and divided them into an iron supplement (n = 13) or placebo group (n = 9) based on initial serum ferritin concentration (24.2 ± 2.9 and 58.5 ± 4.0 µg × l-1, respectively). Exercise for these women consisted of a 35-min run (80 % V˙O2peak) and was performed at week 0 (WK0), after two weeks of intensified training (WK2) and after eight weeks recovery training (WK10). The eight weeks recovery training were concomitant with subjects taking iron supplements or placebo in a double blind fashion. Concentrations of serum ferritin, serum iron and total iron binding capacity were assessed pre-exercise and complete blood count, natural killer cell activity (NKACT), and cell surface markers for CD3+, CD4+, CD3+,CD8+, CD3-, CD16+, CD56+ cells were determined both pre- and post-exercise.The study concluded that NKACT and NK cell numbers were lower in subjects with greater body mass and lower iron stores ($p < 0.05$), but were not significantly altered after two weeks of intensified training or when serum ferritin levels increased. This conclusion is supported by the following data. Serum ferritin concentrations were significantly ($p < 0.05$) increased on WK10 compared to WK2 (time effect). NKACT (%lysis) and NK cell number was lower ($p < 0.05$) at WK0 for supplement (42.9 ± 1.9 % and 305.5 ± 15.0 × 106 × l-1, respectively) compared to placebo groups (50.9 ± 2.0 and 406.1 ± 25.6, respectively). Two weeks of intensified training did not alter indices of host defense. NKACT or NK cell activity was proved to be decreased in women runners with IDA, however the connection is not strong and needs further research.

Using a newly developed in vivo erythrophagocytosis assay, researchers demonstrated that activated cells of the myeloid phagocytic system display enhanced erythrophagocytosis causing acute anemia (*Cnops, J et al 2015*). The study found that NK, NKT and CD8+ T cell-derived IFNγ is a critical mediator in trypanosomosis-associated pathology, driving enhanced erythrophagocytosis by myeloid phagocytic cells and the induction of acute inflammation-associated anemia. Acute inflammation- associated anemia related to IDA because in acute inflammation- associated anemia Inflammation prevents your body from using stored iron to make enough healthy red blood cells, leading to IDA. Results indicate that IFNγ plays a crucial role in the recruitment and activation of erythrophagocytic myeloid cells, as mice lacking the IFNγ receptor were partially protected against trypanosomiasis-associated inflammation and acute anemia. NK and NKT cells were the earliest source of IFNγ during T. b. brucei infection. Later in infection, CD8+ and to a lesser extent CD4+ T cells become the main IFNγ producers. Cell depletion and transfer experiments indicated that during infection the absence of NK, NKT and CD8+ T cells, but not CD4+ T cells, resulted in a reduced anemic phenotype similar to trypanosome infected IFNγR-/- mice. This study proves that the dysfunction of NK cells' cytotoxicity, production and overall

functionality lead to myeloid cell activation which leads to increase in acute inflammation- associated anemia and IDA.

Consequently, the effect of in vitro interferon stimulation on non immune- and immune-spleen natural killer cell activity was studied in iron-deficient rat pups (*Lockwood, J. F., et Sherman, A. R. 1988*). Dams were fed 6, 12 or 250 mg Fe/kg diet during gestation and lactation. Approximately one-half of the 17-d-old pups were injected intraperitoneally with 105 plaque-forming units of vaccinia virus. Four days later, nonimmune and vaccinia-immune pups were killed. Spleen lymphocyte suspensions were prepared and plated with or without rat alpha/beta interferon for 2 h at 37°C. Washed lymphocytes were combined with 51 chromium-labeled YAC-1 target cells and co-cultured for 4 and 16 h at 10 and 50:1 effector-to-target ratios. This study came to the conclusion that Impaired spleen natural killer cell activity in iron-deficient neonates may be due to a limited capacity for stimulation by interferon. Which proves that NK cell activity can be impaired due to IDA. This conclusion is supported by the fact that in general, interferon stimulation increased natural killer cell activity above baseline in iron deficient pups. Analysis of variance comparison among groups showed that interferon was incapable of restoring natural killer cell activity of iron-deficient pups to the levels observed in control pups. In ID rat pups, natural cell activity was proved to be affected because of IDA related lack of stimulation by interferon. This study paves ways for potential therapeutic treatment of IDA related lack of immunity through interferon stimulation.

In a study titled ” Iron metabolism dictates NK cell function” researchers assessed how cellular metabolism relates to proliferation and effector maturation of naïve (NV) vs. cytokine-enhanced (CE) NK cells. Through a literary analysis. The study came to the conclusion that regulating CD71 in the context of pseudo iron deficiency results in increased proliferation of CE NK cells – a concept with potentially broad relevance when aiming to improve proliferation of engineered immune cells. Glycolysis was similarly induced and equally required for NV and CE NK cells to proliferate and acquire effector function. By contrast, upregulation of CD71 was a key discriminating factor between in vitro activated NV and CE NK cells, with distinctly higher cell surface expression on stimulated CE NK cells. Differential expression of CD71 translated into an increased capacity of CE NK cells to take up transferrin/iron, and was associated with higher proliferation rates. CD71-mediated iron uptake was a prerequisite for activation-induced NK cell proliferation also in vivo. In CE NK cells upregulation of the iron regulatory proteins 1 and 2 (IRP1/2) selectively created a pseudo iron deficient state. This cellular state enabled increased translation of CD71 and hence proliferation of activated CE NK cells. The study concludes that regulating the metabolism of cytokine enhanced NK cells during IDA or pseudo IDA resulted in increased proliferation. Therefore increase in cytokine signalling proteins during IDA increases NK proliferation, and IDA results in increase of cell activity (cytokines) which results in increase in NK cell production.

Moreover, in order to determine if changes in systemic iron status regulate intra-hepatic lymphocyte responses. Researchers used a murine model of lymphocyte-mediated acute liver inflammation induced by Concanavalin A (ConA) injection employing mice fed with an iron-deficient (IrDef) or an iron-balanced diet (IrRepl) (*Bonaccorsi-Riani et al 2015*). The mild iron deficiency induced by the IrDef diet did not significantly modify the steady state immune cell repertoire and systemic cytokine levels, it significantly dampened inflammatory liver damage after ConA challenge. These findings were associated with a marked decrease in T cell and NKT cell activation following ConA injection in IrDef mice. The decreased liver injury observed in IrDef mice was independent from changes in the gut microflora, and was replicated employing an iron specific chelator that did not modify intra-hepatic hepcidin secretion. Furthermore, low-dose iron chelation markedly impaired the activation of isolated T cells in vitro. These results suggest that small changes in iron homeostasis can have a major effect in the regulation of intra-hepatic lymphocyte mediated responses. IDA induced by diet can modify cytokine levels that lead to decrease in NK cell activation.

Researchers analysed lymphocyte subsets and NK-cell activity in the peripheral blood of 21 patients with iron deficiency anemia and found lower NK cell activity and population in patients with IDA. (*Santos, P., et Falcão, R. 1990*). The results of this study showed that the mean number of total lymphocytes, CD3 and CD4 subsets, and B lymphocytes were decreased in patients with IDA. However, the NK-cell activity as measured by specific cytotoxicity and cytotoxic capacity was also decreased in patients with IDA. Yet, studies performed in 16 of these patients after the treatment revealed the recovery of these parameters except the decreased NK-cell activity. Proving that IDA leads to loss in NK cell activity, activation and function.

However a literature review (*Farthing, M. J. G 1989*) of animal and human studies concerning the impact of iron deficiency on immune function in vivo disagrees with all previous findings, and indicates that in many instances there is no firm consensus of opinion as to the relationship between iron status and immunity. This is because of the lack of control in environments human studies take in place, leading to confusion with macronutrients, micronutrients ETC and whether or not other factors are skewing up results. Furthermore, the study also suggests that while immune function abnormalities have been detected, their biological and clinical relevance is uncertain. Available studies suggest that iron deficiency may impair NK cell function and T lymphocyte function, particularly DTH responses and mitogen-induced proliferation. However, humoral immunity, including immunoglobulin production and complement levels, appears unaffected. The only consistent abnormality in non-specific immunity is reduced bactericidal activity of polymorphonuclear leukocytes, though its clinical significance remains unclear.

Moreover a study that analysed decreased TIM-3 expression of peripheral blood natural killer cells in patients with severe aplastic anemia (*Zhang, T et al 2017*) found that TIM-3 expression is decreased on peripheral blood NK cells and CD56dim NK subsets in SAA untreated patients. The decrease in TIM-3 relates to NK cells and its correlation with IDA, because Tim-3 serves as a marker for NK-cell activation or maturation and when cross-linked can suppress NK cell–mediated cytotoxicity, and untreated SAA results in IDA, and treated SAA results in overload. Researchers enrolled twenty-two (twelve males, ten females) patients with a median age of 19.5 years (range 7–65) in the study. All patients were diagnosed in the Hematology Department of General Hospital Tianjin Medical University from August 2014 to August 2015, including eleven newly diagnosed cases (six males and five females, median age of 19 years, range 9–65) and eleven remission cases (six males and five females, median age of 21 years, range 7–49) after IST. The researchers detected the TIM-3 expression on the surface of total NK cells and NK subsets by FCM (Fig. 3A). The TIM-3 expression on peripheral blood NK cells in SAA untreated patients (63.57 ± 12.14%) was significantly lower than that in SAA remission patients (75.88 ± 12.83%, $P < 0.05$) and normal controls (85.62 ± 9.03%, $P < 0.01$). There was no difference between SAA remission patients and normal controls (Fig. 3B). The TIM-3 expression on CD56dim NK cells in SAA untreated patients (66.41 ± 11.74%) was lower than treated patients.Untreated patients are the ones with IDA, and patients who were untreated had less TIM-3 expression in NK cells and therefore had less cell activity.

Another study found that IDA significantly reduces tumor incidence in DMBA‐treated rats by mechanisms other than NK cell cytotoxicity, TNF‐activity, and food restriction.Mammary tumor incidence, natural killer (NK) cell activity, and tumor necrosis factor‐α (TNF‐α) activity were measured in iron (Fe)‐deficient and iron‐replete rats treated with the carcinogen 7,12‐dimethylbenz[a]anthracene (DMBA). Female weanling rats were fed AIN‐76 diets: the iron‐deficient group was fed 5 mg Felkg diet; the control group was fed 50 mg Felkg diet; the food‐restricted group was fed 50 mg Felkg diet in the amount consumed by the iron‐deficient group; and the replete group was fed 5 mg Felkg diet for 45 days and then 50 mg Felkg diet. After six weeks of feeding, the rats were given a single intragastric dose of DMBA.Feeding the iron‐deficient diet for 20 weeks reduced hematocrit, hemoglobin, liver iron, and tumor iron values and increased spleen weight. Dietary iron repletion for 14 weeks reversed these effects of iron deficiency. Splenic NK cell cytotoxicity against YAC‐1 cells was highest in the control group. Repleting rats with 50 mg Felkg diet corrected iron deficiency but did not restore NK cell

cytotoxicity. No significant differences in macrophage TNF‐α bioactivity were found among groups. Cumulative tumor incidence over all weeks was lowest in the iron‐deficient rats. Iron repletion during the promotion phase of tumorigenesis attenuates the protective effects of iron deficiency. Food restriction to the extent present in the iron‐deficient group did not protect against tumorigenesis. The iron‐deficient group had the lowest tumor burden and delayed onset of tumors. The part of this study that proves the fact that IDA results in a lack of NK cell activity is that rats with ID were less likely to be able to kill tumors, and the ID group had the lowest tumor burden and delayed onset of tumors. This inability to kill tumors proves that the NK cells in these rats were not being activated properly, and not functioning correctly. Moreover such a decrease in activity can also prove connections to decrease in cytokine production.

Researchers in a 1986 study investigated the natural killer (NK) activity of both fractionated (Percoll density gradient) and unfractionated mononuclear cells from patients with beta-thalassemia major who are iron overloaded as a consequence of chronic transfusion therapy. To determine whether or not found decrease in NK activity could be related to iron overload, researchers also preincubated patient effector cells with desferrioxamine (DFO) or 2,3-dihydroxybenzoic acid (DHB) for 6 hr before addition of K562 targets. (iron chelating agents) (*Akbar, A. N et al 1986*,). Patients were found to have significantly decreased NK activity against K562 targets at all effector:target ratios tested (p less than 0.001). Both splenectomized and nonsplenectomized patients had normal proportions of Leu-11b-staining (NK) cells. Due to normal to elevated absolute white cell and lymphocyte counts, a change in the absolute number of NK cells could not account for the decreased killing. \Moreover, the decrease in NK activity was transfusion related (r = -0.603, p less than 0.005). When added, iron-chelating agents consistently increased the NK activity of cells from thalassemia patients. DHB had the greater effect, being able to increase patient NK activity to virtually normal levels. Preincubation of cells from normal controls with DHB caused only a slight increase in NK activity, and similar treatment with DFO had little or no effect. When target cells were preincubated with the chelating agents before addition of either normal or patient effector cells, no change in cytotoxicity was seen, demonstrating that the chelating agents act at the effector cell level. If the chelating agents were saturated with iron prior to preincubation with the effectors, no increase in the cytotoxicity of thalassemic NK cells was observed. Results of this study indicate that thalassemia patients have a reversible, transfusion-related decrease in NK function which may arise as a consequence of iron overload. This relates to the overall topic of NK cell activity in relation to IDA because it has been proved that a decrease in NK cell activity is due to too much iron, which means that a decrease in iron could lead to beneficial effects towards NK cell activity.

According to a book titled iron and the immune system (*Brock, J. H. 2018*) total lymphocyte numbers are usually unchanged in iron deficiency, however since decreased mitogen responses of lymphocytes from iron-deficient mice are found to be due to the low saturation of serum transferrin, It can be assumed that NK cell activity is affected by IDA especially because mitogens help activate NK cells, leading to increased proliferation, cytotoxic activity, and cytokine production. No specific data is given for this claim, however decreased mitogen response is essential for NK cell activation, recognition, and communication through cytokines. However, according to an entry into the journal of nutrition via literature review, that cited 73 articles. Inadequate nutriture of zinc, copper and iron alter immunocompetence in humans and experimental animals. No further data nor explanation was given for this claim. However immunocompetence is correlated with NK cell activity, which can prove NK cell activity is negatively affected by IDA. However the evidence for this claim is not very strong.

A study that assessed maternal stress during pregnancy predispositions for iron deficiency in infant monkeys impacting innate immunity, came to the conclusion that for monkey babies who are at 4–6 months of age, the emergence of IDA significantly accentuated an effect of prenatal stress on natural killer cell activity (*Coe, C. L et al 2017*). Proving that the existence of IDA in a body decreases and stresses NK cell activity. The influence of maternal stress during pregnancy on the postpartum iron status and immune maturation of infants was

investigated by researchers in a nonhuman primate model. Forty infant rhesus monkeys were generated from two types of disturbed pregnancies, early or late gestation stress, and compared with 24 undisturbed controls. Prenatal stress increased the prevalence and magnitude of iron deficiency (ID) as the infants' growth-related demands for iron exceeded dietary intake from breast milk. By 2 months, infants from disturbed pregnancies, especially those with ES (early stress) conditions, showed reduced NK cell cytolytic activity against all targets ($F_{2,61} = 4.03$, $p < 0.023$). By 6 months, the difference was more pronounced, with IDA (Iron Deficiency Anemia) emerging. Cytolytic activity against Raji and Daudi cells was further reduced in ES infants, and marginally lower against K562 cells, while controls showed increased killing. A significant interaction was found between prenatal condition and target type ($F_{4,122} = 3.18$, $p < 0.016$).These differences were not due to lymphocytopenia or reduced NK cell numbers, as CD16+CD56+ NK cell percentages remained stable (13.9–18.9%). Statistical modeling showed MCV correlated with reduced NK cytotoxicity, especially against Raji and Daudi cells. A greater MCV decline was associated with more significant reductions in NK activity at 6 months. Infants with lower birth weights and higher postnatal growth rates during the first two months were more likely to experience large decreases in both MCV and NK activity. Lower MCV levels were still linked to reduced NK lysis, even after accounting for growth factors, indicating that prenatal stress contributed to these changes. Succinctly, baby monkeys who were born with IDA were much more likely to develop decreased NK cell activity and function.

Another study that was conducted to examine the relationship between iron status and NK activity in highly conditioned female athletes, found that a significant relationship between iron status and resting immune function including NK cells could not be established. Exercise training may affect NK activity; however, the influence of iron status on immune function requires further evaluation (*Braun, W. et al 2000*). Ten collegiate female swimmers (SWM) and 9 inactive females (SED) participated in this investigation. Resting blood samples were obtained and analyzed for serum iron and ferritin. NK activity (% lysis) was determined using a whole blood method (51Cr release assay). No significant relationship was found between iron and NK activity ($r = 0.55$, $p = .09$), nor between serum ferritin and NK activity ($r = 0.33$. $p = .35$) for SWM. ANOVA revealed significantly greater NK activity for SWM (51.63 ± 15.79%) versus SED (30.34 ± 13.67%). Serum ferritin levels were not significantly different between SWM (20.38±8.62Ηg · ml−1) and SED (16.79±10.53Ηg · ml−1), nor were iron values different between groups (16.54 ± 2.17 μmol · L−1 SWM; 11.92 ± 2.61 μmol · L−1 SED). The results of this study indicate that there is no correlation between IDA and NK cell activity, nor is there a significant correlation found between resting exercise training and NK cell activity.

Similarly, a study focused on assessing the in vitro cytokine production in patients with iron deficiency anemia (*Bergman, M., et al 2004*), found that the removal of iron stores from the body causes a decrease in T-cell and NK cell proliferation and differentiation with subsequent cytokine secretion. The results of the present work indicate that PBMC from patients with IDA secrete less IL-2 than cells from healthy controls. IL-2 plays a crucial role in the activation and proliferation of NK cells. A reduction in IL-2 would impair NK cell function, as this cytokine is essential for enhancing NK cell cytotoxicity and supporting their activation. In order to come to this conclusion, researchers analysed the in vitro production of interleukin (IL)-1β, IL-2, IL-6, IL-10, and tumor necrosis factor alpha (TNFα) by peripheral blood mononuclear cells (PBMC) from 20 patients with iron deficiency anemia (IDA) and examined the samples before and after iron supplementation and compared to them values obtained for PBMC from healthy controls. Researchers found that a significant decrease in IL-2 production was observed in IDA patients, whereas the secretion of the other cytokines did not differ from that of controls. Addition of iron to the culture medium did not affect the secretion of IL-2 and IL-1β, but caused an increase in IL-6, IL-10, and TNF-α production. These cytokines are heavily essential not only for cytotoxicity,yet also for NK cell activity, function, recognition and communication with other lymphocytes. Decrease in IL-2 due to IDA is detrimental to NK cell activity, while the increase in IL-6, IL-10, and TNF-α production after iron supplementation proves that the cytokines IL-6, IL-10, and TNF-α are heavily dependent on iron, and IDA would detrimentally affect cytokines IL-6, IL-10, and TNF-α production thus negatively impacting NK cell activity.

Furthermore another study focused on assessing the effect of iron loading on peripheral blood lymphocyte subsets and on circulating cytokine levels in Iron-Depleted hemodialysis patients receiving erythropoietin (*Tsouchnikas, I 2007*) found interesting results. The aim of this study was to evaluate the effect of iron load on peripheral blood lymphocytes subsets and on circulating cytokine levels in HD iron depleted patients, treated with EPO. Researchers studied 19 stable adult HD patients, 12 males, with a mean age 59 ± 11 years and mean HD duration 24 ± 14 months. All patients were iron deficient and were treated with unchanged EPO dose for the last 4 months before entering the study. The administered dose of iron was infused intravenously (1,000 mg iron sucrose) in 10 doses, during 10 consecutive HD sessions. Patients were screened before the commencement of the HD session on two occasions, once prior to the first dose of iron and 2 days after the 10th dose. Hematocrit (Ht), hemoglobin (Hb), iron, serum ferritin, transferrin saturation, interleukin (IL)-2, IL-4, IL-10, interferon-γ and tumor necrosis factor-α were measured. Major lymphocyte subsets (CD3+, CD19+, CD4+, CD8+, CD16+/56+, CD3+CD16+CD56+) and the ratio CD4+/CD8+ were also determined by two-color immunofluorescent analysis using flow cytometry. Relevant data from this study list as the following. Hb, transferrin saturation and ferritin increased significantly at the end of the study 11.2 ± 0.9 to 11.6 ± 0.8 g/dl, $p < 0.005$, 17.5 ± 6.9 to 23.0 ± 10.8 %, $p < 0.05$, and 70 ± 43 to 349 ± 194 μg/l, $p < 0.005$, respectively. IL-2 also increased significantly 27.8 ± 15.2 to 38.9 ± 12.8 pg/ml, $p < 0.05$. After iron load there was no significant change to the major lymphocyte subsets examined but a significant increase of the percentage and number of T lymphocytes with positive natural killer receptors (NKR T) cells was observed, 5.1 ± 3.7% to 6.3 ± 3.46%, $p < 0.05$, and 76.4 ± 40 to 101.5 ± 48 cells/μl, $p < 0.005$, respectively.Leading to the conclusion that iron load in iron-deficient EPO-treated HD patients did not produce any changes in major lymphocyte subsets in peripheral blood, but it resulted in a significant increase of NKR+ T cells, a subpopulation important for local immune responses. Iron load for a relatively short period improved anemia of HD patients and influenced the levels of the circulating IL-2, which may regulate factors affecting the survival of patients. An increase in IL-2 production could have a positive impact on NK cells, as IL-2 is a critical cytokine for NK cell activation, proliferation, and cytotoxicity. Leading to the conclusion that iron is necessary for IL-2 production, and increase in iron leads to increase in IL-2 while IDA results in lack of IL-2, which is significant because IL-2 is a cytokine that is quite necessary for key NK cell activation, functions and communication- thus IDA negatively impacts NK cell activity.

Furthermore, To determine whether reduced IFN-γ contributes to impaired immunity, researchers measured IFN-γ in supernatants of activated (2.5 μg/ml concanavalin A, 50 ng/ml anti-CD3 antibody) spleen cells from control (C), iron-deficient (ID), pair-fed (PF), and iron-replete mice for 3 (R3) and 14 days (R14) (11–12/group). Considerately, the diet of the low iron (5 ppm) mise and control (50 ppm) mice had identical composition, except for iron composition (*Kuvibidila, S. R., 2010*). These methods were utilised in order to assess how iron deficiency, but not underfeeding reduces the secretion of interferon-gamma by mitogen-activated murine spleen cells. The study found that mean indices of iron status after 51 days of feeding were as follows: $C = PF \approx R14 > R3 > ID$ ($p < 0.01$). Iron deficiency, but not pair feeding reduced IFN-γ concentration in mitogen-treated cells by 30–43% ($p < 0.05$); iron repletion improved it. Reduced IFN-γ was not simply due to differences in IL-12 (IFN-γ inducer), percentage of CD3+ T cells, or impaired cell proliferation because these indices were not always decreased. It was likely due to a defect in T cell activation that leads to IFN-γ gene expression. IFN-γ positively correlated with indicators of iron status, body, and thymus weights ($r = 0.238–0.472$; $p < 0.05$). Leading to the conclusion that IDA impairs IFN-γ secretion by mitogen-activated murine spleen cells, IFN-γ (Interferon-gamma) plays a key role in NK cell activation, and cytotoxicity. The cytokine enhances NK cell cytotoxicity by increasing the production of cytotoxic proteins like perforin and granzyme. Moreover IFN-y plays a big role in NK cell communication and activation. IDA impairing IFN-Y secretion is compelling evidence that IDA negatively affects NK cell activity.

A literature review focused on the role of iron in chronic inflammatory diseases: from mechanisms to treatment options in anemia of inflammation (*Marques, O et al 2022*), found that increase in NK cell activity due to inflammation can result in increased iron accumulation and retention. Increase in cytokines due to inflammation also leads to influence in NK immune cell responses due to altered available iron. Evidence for this claim lies in the fact that macrophages accumulate iron in response to cytokines and PAMPs during inflammation, which decreases FPN-mediated iron export and further contributes to iron retention. Proinflammatory cytokines like IL-6, IL-1β, and others upregulate hepcidin which affects iron homeostasis and indirectly influences immune responses by altering the iron available to immune cells. Proving that inflammation related increased NK cell activity leads to increased iron accumulation in the blood, proving that NK cell activation relies on iron in order to function and IDA would result in a lack of NK cell activity.Moreover increase in cytokine responses due to IDA leads to increased NK cell response and activity, leading to the conclusion that connections between IDA and NK cell activity may just be a circle.

The objectives of an experiment published in the journal of animal science were to determine the effects of production system and genotype on pig performance and health and to determine whether C-15-405 pigs reared outdoors or indoors needed supplemental iron or whether they would receive enough environmental iron, and how the lack of supplemental iron may impact pig Hb and immunity. Sows were bred, gestated, farrowed, and lactated in either an intensive indoor or an intensive outdoor production system. The three dam genotypes of pigs used in each environment were PIC Camborough-15 (C-15), PIC Camborough Blue (CB), and Yorkshire × Landrace (YL). All pigs received 100 mg of iron dextran at day 3 of age. Indoor and outdoor pigs received either no supplemental iron, 100 mg, or 400 mg of iron dextran on day 3 of age *Kleinbeck, S. N., et McGlone, J. J.1999.* The study found that pigs raised in the outdoor unit had higher blood hemoglobin (Hb) concentrations on d 28 of age than pigs raised indoors (11.5 ± .22 vs 8.16 ± .26 g/dL, $P < .0001$). Outdoor-reared pigs also had more white blood cells (WBC) on day 3 than indoor-reared pigs (9.7 ± .38 vs 8.04 ± .38 cells/μL × 103, $P < .05$), but outdoor pigs had fewer WBC on d 28 of age than indoor-reared pigs (9.8 ± .5 vs 11.1 ± .45 cells/μL × 103, $P < .05$). Genetic lines did not differ in plasma immunoglobulin G (IgG) concentrations at 3 or 28 d of age. Environment and age influenced pig Hb levels and WBC numbers. Blood percentage neutrophils and neutrophil: lymphocyte ratio were lower ($P < .05$) indoors, and natural killer cell (NK) activity was greater ($P < .05$) among indoor- than outdoor-reared pigs (NK % cytotoxicity: 15.6 ± 2.3 vs 9.7 ± 2.3). Outdoor-reared pigs that received no injected iron had similar Hb at d 28 of age as indoor-reared pigs that received 100 mg of iron dextran (11.1 ± .36 vs 10.7 ± .4 g/dL, $P = .59$). Leading to the overall conclusion that (NK) activity was greater ($P < .05$) among indoor- than outdoor-reared pigs , However outdoor pigs normally had more iron, and were not deficient in iron, WBC, or lymphocyte count. Which is why these results indicate that IDA leads to an increase in NK cell activity.

A literature review completed in 2005 (*Weiss, G. 2005*) came to the conclusion that upon activation, T cells, NK cells and macrophages produce a number of cytokines which directly or via secondary formation of acute-phase proteins or radicals then influence iron metabolism by transcriptional and posttranscriptional mechanisms affecting cellular iron uptake, transmembrane iron transport, iron recirculation, and iron storage, as well as iron absorption. Proving that NK cell released cytokines can cause IDA and problems in iron absorption and storage. Evidence for this claim lies in the fact that the paper claims that cytokines, immune-cell-derived radicals, and acute-phase proteins affect the regulation of iron homeostasis at different levels ranging from transcriptional interference with iron genes to modulation of iron transport capacities of transmembrane iron channels. Therefore, since NK cell released cytokines impact iron homeostasis, malfunction in NK cell activity can result in both IDA and excess iron accumulation.

According to a literature review titled “Absolute and functional iron deficiency: Biomarkers, impact on immune system, and therapy”. There is a very vague connection between IDA and NK cells, and this article also proved that NK cells have immune memory. Connection between the two is established, however the depth of connection

and whether or not it is positive or negative is not stated. Slight evidence is given for this, and that aforementioned evidence is stated as follows. ID can affect vaccine response, immune memory and other immunity-related factors. ID impacts not only red-blood cells but also immune system cells (NK cells) , highlighting its importance in global health and immune-related comorbidities. However the extent to the connection and what exactly the connection is, is not given due to lack of access. Furthermore another literature review (*Huntington, N. D. 2023*) concludes that transcription factor IRF4 controls iron acquisition and is upregulated following NK cell activation, This indicates that when NK cells activate, they consume iron and therefore high NK cell activity results in lower iron and lower NK cell activity results in higher iron, and vise versa. The evidence behind this claim lies in a study included in this literature review that finds that IRF4 is upregulated following natural killer (NK) cell activation and is required for the differentiation and expansion of virus-specific NK cells by controlling nutrient acquisition, including iron uptake. Thus when NK cells activate, they consume iron and therefore high NK cell activity results in lower iron and lower NK cell activity results in higher iron, and vise versa (probably due to usage).

A series of food restriction experiments on weanling C57BL/6 mice (*Spear, A. T. 1992*) done in order to access cytokine activities and natural killer cell cytotoxicity in response to food restriction and iron deficiency in rodents. Found that NK cell activity and cytotoxicity appears to be negatively impacted by more severe food restriction, while moderate restriction preserves NK cell function. Cytokines like IL-1, IL-2, IFN-α, and TNF-α show varied responses to food restriction, with some being more sensitive to changes than others.

Weanling C57B16 mice who were food restricted by 20% for 5 wk exhibited conserved spleen:body weight ratio, conserved natural killer (NK) cell activity and greater interleukin-1 (IL-1) activity; while mice food restricted by 40% or 60% had reduced spleen:body weight ratio, reduced NK cell cytotoxicity, NK cell activity and conserved IL-1 activity compared with mice fed ad libitum. Interleukin-2 (IL-2) production, interferon (IFN) a and tumor necrosis factor (TNF) a activities were not different between the carbohydrate and food restricted mice. The effect of food restriction on IL-2 production was dependent upon mitogen stimulation: basal IL-2 production was higher in the food restricted mice and mitogen-stimulated IL-2 production was lower compared with mice fed ad libitum. After 12 weeks of food restriction from d 35 to d 119 of age in C57B16 mice, the NK cell response to an in vivo stimulant was impaired, but IFN a activity was conserved compared with feeding ad libitum. Twelve weeks after carcinogen administration, NK cell cytotoxicity, IFN a and TNF a activities were not different between the food restricted rats and rats fed ad libitum. Changes in production of Cytokines like IL-1, IL-2, IFN-α, and TNF-α, prove that IDA results in decreased NK cell activity, while specified decreases in NK cell activity in the article also prove that point simultaneously.

Another study, done through use of an experimental model of human zinc deficiency based on zinc-deficient dwarfs from the Middle East, aids the conclusion that zinc deficiency can contribute to iron deficiency anemia, as zinc plays a crucial role in iron metabolism and erythropoiesis (red blood cell production), and deficiencies often occur together. There is no confirmation of IDA in the article, however zinc deficiencies result in decreased activity of NK cells and decreased lymphocyte count should be noted, as iron deficiencies often follow zinc deficiencies.The study reported that zinc deficiency resulted in decreased thymulin (a thymopoietic hormone) activity in Th1 cells, decreased mRNAs of IL-2 and IFN-gamma genes, and decreased activity of natural killer cells (NK) and T cytotoxic T cells. The effect of zinc deficiency on thymulin activity and IL-2 mRNA was seen within eight to twelve weeks of the institution of zinc-deficient diet in human volunteers, whereas lymphocyte counts decreased in 20 weeks and plasma zinc decreased in 24 weeks after instituting zinc-deficient diet. Decreased generation of IL-2 and IFN-gamma and no effect in Th2 cell function was also observed. Thus, zinc deficiency resulted in an imbalance of Th1 to Th2 function resulting in decreased cell-mediated immunity. Therefore both the listed decrease in cytokine production and noted decreased activity of NK cells should lead to the conclusion that zinc related IDA results in decrease in activity of NK cells.

Genetically determined Hemophagocytic lymphohistiocytosis (HLH) also referred to as familial hemophagocytic lymphohistiocytosis, commonly manifest in infants or in young children and are characterized by genetic abnormalities of natural killer (NK)-cell function due to gene mutation of HO-1. These abnormalities of NK cell function lead to abnormal inflammatory cytokine levels and bilirubin synthesis caused IDA (microcytic), (*Greil, J, 2016*). These findings were collected through analysis of multiple patients who have Hemophagocytic lymphohistiocytosis (HLH) and the gene mutation of HO-1. These findings of course lead to the conclusion that HLH genetic mutations of HO-1 that cause NK cell activity abnormalities lead to abnormal inflammatory cytokine levels and bilirubin synthesis causes IDA (microcytic). The findings of this study relate to IDA because the conclusion that HLH genetic mutations of HO-1 that cause NK cell activity abnormalities lead to abnormal inflammatory cytokine levels and bilirubin synthesis caused IDA (microcytic) means that abnormal NK cell activity leads to IDA.

Furthermore, A researcher analysed another researchers project (*Bell, H. N 2024*)where deferiprone, an iron chelator, was used to reduce iron in ovarian cancer cells, triggering the cGAS/STING pathway and boosting type I IFN production. This activated IL-15 in dendritic cells, which recruited and activated NK cells to enhance their antitumor activity. The treatment also synergized with cisplatin chemotherapy to reduce ovarian cancer metastasis and improve survival in murine models. The findings list as follows. Iron chelator deferiprone directly reprograms ovarian cancer cells to produce increased type I IFN and enhance NK cell-mediated immunity. NK cells produce perforin and granzyme, manufacture high levels of IFNγ and TNFα, and are highly cytolytic. Type I IFN is critical for the effective activation and antitumor function of NK cells. Type I IFN induces IL15 expression by dendritic cells (DC), which then stimulates NK cell activity and recruitment. Iron chelation did not induce cytotoxicity in tumor cells alone, but rather robustly increased the number of NK cells in the peritoneal cavity. NK cell ablation using antibody therapy did not impact the survival of vehicle or cisplatin-treated mice but substantially curtailed the therapeutic impact of deferiprone. RNA sequencing analysis revealed that alongside iron-regulated genes, deferiprone repressed immunosuppressive TGFβ and IL10 while upregulating tumor cell transcripts involved in type I IFN signaling and NK-cell activation. deferiprone-induced increase in mitochondrial DNA triggers the cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS)- stimulator of IFN genes (STING) pathway, leading to the phosphorylation of IRF3 and increased expression of the type I IFN system. Iron chelation with deferiprone activates the DNA damage response in parallel through immediate phosphorylation of CHK1, IL15 can drive NK-cell proliferation and NK cytotoxicity (4). Type I IFN induces expression of IL15 in DCs, which in turn stimulate neighboring NK cells (4). Deferiprone indeed increased the proportion of tumor-associated DCs expressing high levels of IL15, and DC depletion reduced NK-cell infiltration at tumor sites. Which leads to the conclusion that iron chelation enhances type I IFN production, promotes NK cell tumor trafficking and activation, and synergizes with chemotherapy drug cisplatin to reduce metastatic ovarian cancer progression in murine models. In other words, removing iron (IDA) promotes NK cell activity and cytotoxicity.

Moreover iron is proven to affect the activation of NK cells, however the specific effects of deficiency vs surplus are yet to be highlighted (ambiguous), (*Terpiłowska, S. 2011*). These findings were developed in a literature review published in the Termedia.pl. After thorough analysis, the authors of this paper came up with the following observations A number of nutrients/ elements have the ability to modulate immune response through the production of antibodies or cytokines (e.g. zinc, selenium, chromium, iron) and may be required for immune cells proliferation or activation (e.g. iron). These elements are also required for functioning of enzymes involved in the antioxidant system (e.g. selenium) of the immune cells. It has been shown that nutrient supplementation may enhance but may also suppress immune function. Which is why the conclusion that iron does affect the

activation of NK cells is accurate, however the results are quite ambiguous due to lack of evidence and lack of access to the full article.

However a literature review, analysis of previous works concerning zinc, selenium, iron, copper, β-carotene, vitamins A, C, and E, and folic acid and its influences on several components of innate immunity point towards the fact that there is no correlation between iron and NK cell activity. However, select micronutrients play an important role in alteration of oxidant-mediated tissue injury, and phagocytic cells produce reactive oxidants as part of the defense against infectious agents. Thus, adequate micronutrients are required to prevent damage of cells participating in innate immunity. Deficiencies in zinc and vitamins A and D may reduce natural killer cell function, whereas supplemental zinc or vitamin C may enhance their activity. The specific effects of micronutrients on neutrophil functions are not clear. Select micronutrients may play a role in innate immunity associated with some disease processes. Which leads to the conclusion that no specific correlations between IDA and NK cells were found, it can be confirmed however that micronutrients including iron are required to prevent damage of cells participating in innate immunity. However deficiencies in zinc, vitamins a and d reduce NK cell function while supplemental zinc or vitamin C may enhance NK cell activity. Even though the findings suggest that iron is necessary to prevent damage to cells, the study also reported that multiple vitamins were necessary for NK cell activity, yet iron was not included in the list.

According to a literature review, that analyzes previous work on the main functions of iron in blood cells and iron-related diseases in humans and highlights the potential of magnetophoresis for diagnosing and treating some of these disorders, iron homeostasis is dependent on NK cell related cytokines, and IDA can hamper immunological process and hemochromatosis results in mass organ failure. These results were found due to the following. Iron serves an important role related to the function of different leukocytes. In inflammation, iron homeostasis is dependent on cytokines derived from T cells, Nk cells and macrophages. Fluctuations of iron content in the body lead to different diseases. Iron deficiency, which is also known as anemia, hampers different physiological processes in the human body. On the other hand, genetic or acquired hemochromatosis ultimately results in iron overload and leads to the failure of different vital organs.Proving that IDA is relative to NK cell activity because iron homeostasis is dependent on NK cell related cytokines, and IDA can hamper immunological process and hemochromatosis (mass iron) results in mass organ failure. In other words, IDA and the diagnosis of IDA is reliant on NK cell related cytokines and thus NK cell activity, concurrently, IDA can hamper NK cell activity and too much organ can result in failure of NK cells due to mass organ death.

The following project that was analysed consisted of a randomized, double-blind, placebo comparative clinical trial aimed to determine the immune-enhancing effects and safety of a nanomaterial with iron and zinc (ALP1018) in healthy adults. Participants who met the inclusion criteria were recruited for this study ($n = 80$) and randomly assigned to either the test group ($n = 40$), which was given Alp1018 in capsule form, or the placebo group ($n = 40$), which was given crystal cellulose capsules of identical appearance, weight, and flavor for 8 weeks. Compared to baseline, natural killer (NK) cell activity (%) increased in the test group after 8 weeks, although there were no changes in the placebo group. Furthermore, in the subgroup analysis of Coronavirus disease 2019 (COVID-19) affected participants, significantly increased NK cell activity was observed in the test group at 4 ($p < 0.05$) and 8 weeks ($p < 0.05$). No significant differences were observed in cytokine levels between the two groups. ALP1018 supplementation appeared to enhance immune function by improving NK cell activity without adverse effects in healthy adults. These observations led to the conclusion that NK cell activity increased when iron and zinc were increased. Which means IDA results in low NK cell activity. Moreover, no significant difference in cytokine levels were observed and changes in blood cell populations, including immune cells, cytokines, and molecular signaling related to NK cell activity, have not yet been studied.

Natural killer cell cytotoxicity, both basal and interferon gamma (IFN gamma)-stimulated, was studied in moderately and severely iron-deficient rats challenged with the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). Female weanling rats were fed ad libitum semi purified diets containing 8, 13 or 42 mg Fe/kg. A pair-fed group was fed the 42 mg Fe/kg diet at the level consumed by the 8 mg Fe/kg group. Following 6 wk of dietary treatment, DMBA-treated rats received a single intragastric dose of DMBA. Dietary treatment was continued. Rats were killed at 1, 4, 8, 14 and 20 wk post-DMBA treatment. Natural killer cell cytotoxicity (both basal and IFN gamma-stimulated) was analyzed (Spear, A. T., et Sherman, A. R. 1992). Feeding the 13 mg Fe/kg diet resulted in lower NK cell activity (P = 0.006) and greater tumor burden (P = 0.045) and tumor incidence. Interferon gamma treatment relieved the lower NK cell cytotoxicity observed in moderate iron deficiency. Feeding the 8 mg Fe/kg diet impaired NK cell activity (P = 0.006), but tumor burden and incidence were less than in moderate iron deficiency. This led to the study conclusion being iron deficiency, particularly moderate iron deficiency, contributed to cancer development and compromised NK cell cytotoxicity and impaired NK cell activity.This IDA, particularly moderate IDA lead to impaired NK cell activity.

In a thesis based study (*Erny, J. J. 2022*) titled MODERATE MALNUTRITION INCREASES NK CELL POPULATION AND INFLAMMATORY ACTIVATION IN THE SPLEEN, C57BL/6 mice were fed a diet consisting of 3% protein deficient in iron and zinc compared to age-matched well-nourished controls fed a 17% protein content diet supplemented with iron and zinc over 4 weeks. Circulating blood leukocytes and splenic lymphocytes were collected. The researchers found that cytokine production was affected by iron. At day 3 p.i, there was a larger percentage of IFN-γ-producing NK cells in the moderate malnourished mice compared to the well-nourished (p=0.003) (Figure 4A). Co-production of inflammatory cytokines was also found to be elevated in the moderate malnourished mice(p=0.045) (Figure 4B). Unlike IFN-γ, neither malnutrition nor infection impacted TNF-α production (Figure 4C). Thus essential cytokines necessary for NK cell function, communication and activation are positively affected by IDA, and are improved by lack of iron.

Overall, most studies point towards correlation between IDA and NK cell activity, with 87.5 percent of studies agreeing with correlation and 12.5 disagreeing with correlation. Moreover, around 70 percent of articles agree there is negative correlation between NK cell activity and IDA, where IDA and decreased iron is correlated with decreased NK cell activity and not increased cell activity. While 24 percent of articles argue that there is positive correlation between IDA and cell activity, where a decrease in iron is related to an increase and positive effect on cell activity. However, 3 articles or 7 percent of articles have resulted in complicated results where there is both positive and negative correlation between NK cell activity and IDA. The first example of this occurrence lies in a literature review (*Marques, O et al 2022*) where the argument was made that proving that NK cell activation relies on iron in order to function means that IDA would result in a lack of NK cell activity. However, an increase in cytokine responses due to IDA leads to increased NK cell activity, leading to the conclusion that connections between IDA and NK cell activity may just be a circle. While another paper (*Weiss, G. 2005)* claims that since NK cell released cytokines impact iron homeostasis, malfunction in NK cell activity can result in both IDA and excess iron accumulation. Yet analysis of another article (*Terpiłowska, S. 2011*) reveals that iron affects the activation of NK cells, however the results are quite ambiguous due to lack of evidence and lack of access to the full article, and the positive or negative effect is not highlighted. Moreover, around 82 percent of articles indicate that iron affects NK cell activity in either positive or negative manner in that specific order, while 8 percent of articles demonstrate the idea that NK cell activity is the variable that affects iron levels, and that iron levels are affected by NK cell activity and not the other way around. Additionally 10 percent of articles provide evidence for both connections, arguing that IDA impacts NK cell activity and NK cell activity impacts IDA and iron levels, and that both coexist together in a loop of interconnectedness. Yet discrepancy between all articles analysed was noticed, as articles that argued for correlation, positive or negative, and articles that argued against correlation between IDA and NK cell activity, both had strong evidence. Consequently, there were many articles arguing both against correlation and with, leading to the gap in agreement to be of considerable size. However a literature

review analysed in this section provides an answer for the gap in agreement and lack of definite answers. This article is a literature review (*Farthing, M. J. G 1989*) of animal and human studies concerning the impact of iron deficiency on immune function in vivo, and this article disagrees with all previous findings, arguing that while immune function abnormalities in relation to IDA have been detected, their biological and clinical relevance is uncertain and there is no firm consensus of opinion as to the relationship between iron status and immunity. The author argues that huge disparity and lack of consensus is because of the lack of control in environments human studies take in place, leading to confusion with macronutrients, micronutrients ETC and whether or not other factors are skewing up results. Yet, Many studies do show strong correlations towards NK cell activity and IDA. Even though each study analysed consists of wildly different circumstances and experimental conditions, and many articles exist to discredit the interconnection between IDA and NK cell activity. Majority of articles do agree with correlation between IDA and NK cell activity and provide much evidence in the form of not only blatantly stated analysis of NK cell activity, yet also investigation of cytokine production, protein synthesis, MHC1 expression, interferon gamma, DEP'S, TNFα production, mitochondrial function, DNA repair and synthesis, epigenetic regulation, sensing of hypoxia, antiviral functions, multicellular signaling pathways and expressions of many other countless cytokines.

Growth

Fundamentally, cell growth according to *wikipedia*, refers to increase in the total mass of a cell, including cytoplasmic, nuclear and organelle volume. While *Science direct* states that Cell growth refers to the process in which tissues and organs increase in size through cell division and enlargement, of which typically involves three stages: cell hyperplasia, hyperplasia-hypertrophy, and hypertrophy, where cells either multiply or grow in size to reach their adult form. This research paper chooses to separate growth and production and have them refer to two separate things, growth in this research paper will always refer to increase in the total mass of a cell, metabolism and overall functional capacity of a cell that has already been created. While production will always refer to the creation of new cells and increase in NK cell numbers. Growth in this research paper will always refer to development of existing cells and not creation of new cells. Specifically considering NK cells, this research paper will focus on metabolic activity, abnormal growth patterns, size, and expansion and survival in the context of inflammation and infection. Succinctly, the key research question for this section is, does iron deficiency impair NK cell growth and metabolic adaptation in all scenarios including those that consider inflammation and infection. Examples of metabolic pathways that support NK cell growth that will be searched for in relation to IDA , include glycolysis, and oxidative phosphorylation, while size can be assessed through measurements and analysis provided by research papers.Furthermore, while NK cells develop from common lymphoid progenitor cells (CLPs) primarily in the bone marrow, also in the liver and thymus, maturation involves stages marked by the expression and loss of specific receptor expressions which are all types of cell surface markers (e.g., CD117, CD122, NK1.1, NKp46, CD49b, CD11b, CD27). Studying IDA's effects on these receptor expressions should shed a light on maturation and growth of existing NK cells, albeit existing yet developing and primarily unfunctional NK cells. Additionally, mature NK cells exhibit high cytolytic activity and IFN-γ production, which means studying cytolytic activity and IFN-y production should also clarify the growth of an NK cell. Succinctly, this part of the paper will study the relation between IDA and NK cell growth, which refers to the development, maintenance, and change of existing cells, which will be studied through analysis of cell size, metabolism, abnormal growth patterns, survival and expansion in the context of inflammation or infection and consideration of receptor expressions, cytolytic activity, and IFN-y production.

Friendly Neighborhood Immunologist. (2021, September 9). *Natural Killer cells | Top 5 ways Natural Killer cells work* [Video]. YouTube. https://www.youtube.com/watch?v=aBDhnZrxAaY

Drbeen Medical Lectures. (2022, November 10). *Summary - Natural killer (NK) cells* [Video]. YouTube. https://www.youtube.com/watch?v=arz-sYRgLnk

Professor Dave Explains. (2023, December 20). *Natural killer cells: the tumor killers* [Video]. YouTube. https://www.youtube.com/watch?v=iATp8DO3RA8

Results in a research study published in the journal of immunology, indicate that thalassemia patients have a reversible, transfusion-related decrease in NK function which may arise as a consequence of iron overload (*Akbar, A. N et al 1986*). Thalassemia can be used to assess NK cell function in relation to IDA because In individuals with thalassemia, particularly those requiring regular blood transfusions, iron overload can occur due to the body's inability to effectively process the excess iron. Thus thalassemia leads to a hemochromatosis effect where it can be assumed iron is involved in and has an opposite and equal reaction to the reaction towards NK cytotoxicity expected of IDA. Researchers in this study investigated the natural killer (NK) activity of both fractionated (Percoll density gradient) and unfractionated mononuclear cells from patients with beta-thalassemia major who are iron overloaded as a consequence of chronic transfusion therapy. To determine whether or not found decrease in NK activity could be related to iron overload, researchers also preincubated patient effector cells with desferrioxamine (DFO) or 2,3-dihydroxybenzoic acid (DHB) for 6 hr before addition of K562 targets. (iron chelating agents). Both splenectomized and nonsplenectomized patients were found to have had normal proportions of Leu-11b-staining (NK) cells. Due to normal to elevated absolute white cell and lymphocyte counts, a change in the absolute number of NK cells or size could not account for the decreased killing even if it was observed. Proving that excess iron does not lead to abnormal proportions or growth in NK cells.

However another study (*Rosch, L. M et al 1987*) argues that protein synthesis in the liver and thymus is not changed or affected by moderate iron deficiency. Yet the study did find that moderate iron deficiency did impact protein synthesis in the spleen. Protein synthesis in the spleen, liver and thymus in severely iron-deficient pups was less than half that of iron-sufficient pups, representing severe decrease in NK cell population and growth. In this study, RNA, DNA and in vitro protein synthesis were measured. Rats were fed diets containing 6 (severe anemia), 11 (moderate anemia) or 250 (iron sufficient) mg iron/kg diet throughout gestation and lactation. On d 2 of lactation, litters were adjusted to contain six pups. On d 12 of lactation, two pups from each litter were immunized with sheep red blood cells (SRBC) and on d 17, tissues were removed for the determination of protein synthesis and evaluation of RNA and DNA contents.In the moderately iron-deficient pups, protein synthesis was lower (30%) in spleen than that in iron-sufficient pups. Protein synthesis in the spleen of the moderately iron-deficient group was higher after immunization with SRBC than in iron-sufficient controls, whereas the severely iron-deficient pups failed to respond. Impaired protein synthesis may be the mechanism responsible for compromised ability to produce antibody in iron deficiency.Protein synthesis is crucial for NK cell function, influencing their metabolism, survival, proliferation, and effector functions, such as cytokine production and cytotoxicity, and thus protein synthesis is essential for growth as well and increase in protein synthesis could lead to growth in the cell, and lack of protein synthesis could lead to decrease in the cell size.

According to another paper titled “Effect of iron deficiency on the stimulation of natural killer cells by macrophage-produced interferon” published in the journal, Nutrition Research (*Hallquist, N. A., et Sherman, A. R 1989*), Spleen natural killer (NK) cell activity and protein synthesis are decreased in iron-deficient rats. IFN production or ability to stimulate NK cells was decreased by iron deficiency, but syngeneic B cells compensated for the decreased activity. IFN plays a role in decreasing NK cell activity during iron deficiency. Data for this claim liss as follows, Interferon (IFN), activates NK cells, production of IFN by macrophages are low in iron-deficient rats causing impaired NK cell activity. Hemoglobin and hematocrit levels were lower in iron-deficient rats than in other rats. NK cell cytotoxicity after incubation with macrophage-produced IFN decreased in iron-deficient rats (13.4±1.5%) compared to control rats (19.0±1.9%), while increased NK activity was found in pair-fed rats (25.5±1.9%) compared to control and iron deficient rats. In order to come to these

results, researchers took weanling male rats (n=9/group) and fed them ad libitum 5 or 37 mg iron/kg diet for eight weeks. Pair-fed rats (n=6) were fed a control diet as consumed by iron-deficient rats. Spleen NK cell activity was measured by Cr-51 release from labeled Yac-1 cells after NK cells were activated by macrophage-produced IFN. Macrophages were stimulated in vitro with poly inosinic:cytidylic acid to produce IFN. The most important piece of information that can be gathered from this study in relation to NK cell growth is the decreased protein synthesis, which proves that the growth of the NK cells in patients with IDA is abnormal.

Furthermore, a literature review completed by (*Beisel, W. 1982*) states that lymphocyte count, In vitro lymphocyte transformation,antibody production, dermal hypersensitivity, chemotaxis, Phagocytosis, bactericidal activity, and metabolic c3 in NK cells are all impacted by IDA. However, analysis of multiple sources through literature review comes to the following data conclusion. Increased susceptibility to infection due to IDA via lymphoid tissues and reduced lymphocyte and NK cell counts, may lead to impaired In vitro lymphocyte transformation. While normal or impaired antibody production may have decreased delayed dermal hypersensitivity and may have increased or impaired chemotaxis, or phagocytosis impaired bactericidal activity. Metabolic c3 may increase. However no exact correlation was found between IDA and NK cell activity.Lymphocyte transformation however, is defined as the in vitro process where lymphocytes are stimulated to proliferate and change in size and appearance in response to an external threat which is exactly the definition of NK cell growth. However, lymphocytes are a cell subtype that include an array of cells, of which are T cells, B cells and NK cells. Consequently, due to the fact that lymphocyte transformation includes multiple cells, a true conclusion cannot be made. However, if one were to assume that lymphocyte transformation refers to NK transformation and results are not altered by other cells that are a part of the lymphocyte subtype. Then the conclusion could be made that since lymphocyte transformation is affected by IDA, NK cell transformation (growth) must also be negatively affected by IDA. Moreover phagocytosis is a cellular process for ingesting and eliminating particles larger than 0.5 μm in diameter, which is a perfect way to measure NK cell growth in relation to IDA. Thus since phagocytosis, bactericidal activity, metabolic c3, and cell transformation are negatively impacted by IDA, IDA leads to decrease in NK cell growth.

In a recent 2024 study a cohort of PWO's, people with obesity who were confirmed to either have or not have IDA were tested on multiple NK cell factors and cytotoxicity (*DeBarra, C et al 2024*). The data collected demonstrates that in response to cytokine stimulation, healthy human NK cells utilize iron to support their metabolic activity and cytokine responses. In a cohort of PWO, The study demonstrates alterations in NK cell metabolism, mitochondrial fitness and cytokine production. Furthermore, upon stratification into PWO with normal iron status versus low iron status,the observed obesity-related defects in NK cell metabolism, mitochondrial fitness and cytokine production are concentrated in the PWO with low-iron status.Leading to the study conclusion that obesity is associated with significant defects in the functionality of human NK cells, especially in the periphery. Dysregulated cellular metabolism has been demonstrated to be a major mechanistic driver of the reported defects. However, how obesity links to defective NK cell metabolism and functionality remains unclear. Yet the most important parts of this study in relation to growth of NK cells is that defects and negative impacts towards cytokine stimulation, metabolic activity, and mitochondrial fitness can be used to prove defects in NK cell growth in relation to IDA.

Moreover according to a 2021 study titled “Metabolic requirements of NK cells during the acute response against retroviral infection”, Iron deficiency profoundly impairs NK cell antiviral functions, leading to increased viral

loads (*Littwitz-Salomon et al 2021*). Iron is very important for essential cellular activities including mitochondrial function, DNA repair and synthesis, and epigenetic regulation and sensing of hypoxia.The antiviral functions of NK cells strongly depend on sufficient levels of iron.NK cells have an increased demand for iron when responding to acute FV infection and that experimental reduction of serum iron levels inhibits NK cell activation and their IFNγ production. However most importantly considering the research topic, smaller NK cell size and lack of killing were also observed in iron deprived cells. In this study C57BL/6 mice were infected with 40,000 Spleen Focus-Forming Units (SFFU) of FV and the viral loads in the bone marrow and spleens were monitored over the course of 28 days. The bone marrow and spleen were analysed due to high viral replication in these organs25. Since FV preferentially infects erythroblasts, monocytes and macrophages but all dividing cells in the spleen and bone marrow can be targets for infection.Iron and Nk cells were tested after the infection. In order to support these claims the researchers provide the following data There was an increase in the uptake of transferrin into NK cells in FV-infected mice compared to NK cells from naive mice. Increased transferrin uptake and CD71 expression were observed in both NK cell populations from the bone marrow and the spleen following infection, suggesting that responding NK cells have an increased demand for iron.CD71 expression and transferrin uptake are mainly represented by by CD27+CD11b+ NK cells. Analysis of NK cell activation revealed a significant decrease of CD69+ NK cells in animals with reduced levels of serum iron upon acute FV infection in the spleen and bone marrow. Smaller NK cell size was also detected in the FV-infected, iron-deprived group compared to FV-infected, vehicle-treated mice. There was also a dramatic decrease of cMyc+ NK cells in mice with low serum iron upon FV infection.NK cells isolated from infected mice with low serum iron levels showed significantly less killing of FBL-3 target cells than NK cells isolated from infected mice with normal serum iron levels. Proving that IDA leads to decrease in growth of NK cells.

According to a narrative literature review concerning biological and Immunological aspects of iron deficiency anemia in Cancer Development (*Zohora, F et al 2021*), Iron Deficiency Anemia (IDA) is a universal health problem and a risk factor for the development of cancer. IDA changes the microenvironment of the human body by affecting both the biological and immunological systems. It increases DNA damage and genomic instability by different mechanisms. Due to IDA'S effects on the innate immune system and cytotoxicity it can be concluded that IDA leads to deterioration of NK cell cytotoxicity that leads to negative effects on the body's cancer detection and treatment. Yet the most influential part of this study that relates to this part of the paper is NK cell growth. Thus the most important data and conclusions, IDA interrupts the oxidative phosphorylation energy metabolism and intestinal Cytochrome-P450 systems. It also disturbs multicellular signaling pathways involved in cell survival and helps in tumor angiogenesis. Moreover, IDA is also responsible for the functional deterioration of innate and adaptive immune systems that lead to immunological dysfunctions against invading pathogens. Changes in the oxidative phosphorylation, energy metabolism and intestinal Cytochrome-P450 systems indicate changes in growth, due to oxidative phosphorylation being the way cells create energy, decrease in oxidative phosphorylation would lead to decrease in growth due to lack of energy, and because energy metabolism is how all functions inside a cell work and because metabolism is growth, IDA leads to decrease in NK cell growth.

In another study, Researchers assessed how cellular metabolism relates to proliferation and effector maturation of naïve (NV) vs. cytokine-enhanced (CE) NK cells, through a literary analysis (*Hess, C ET al 2021*). They found that regulating CD71 in the context of pseudo iron deficiency enabled increased proliferation and maturation of CE NK cells – a concept with potentially broad relevance when aiming to improve maturation of engineered immune cells.Upregulation of CD71 was a key discriminating factor between in vitro activated NV and CE NK cells, with distinctly higher cell surface expression on stimulated CE NK cells. Differential expression of CD71 translated into an increased capacity of CE NK cells to take up transferrin/iron, and was associated with higher proliferation rates. CD71-mediated iron uptake was a prerequisite for activation-induced NK cell proliferation and

maturation also in vivo. In CE NK cells upregulation of the iron regulatory proteins 1 and 2 (IRP1/2) selectively created a pseudo iron deficient state. This cellular state enabled increased translation of CD71 and hence proliferation of activated CE NK cells. Thus a pseudo iron deficient state created through upregulation of iron regulatory proteins 1 and 2 (IRP1/2) resulted in increase in NK cell maturation and proliferation.

Decreased TIM-3 expression of peripheral blood natural killer cells in patients with severe aplastic anemia were analysed by researchers and published in the journal *Cellular Immunology* (*Zhang, T et al 2017*).The researchers detected the TIM-3 expression on the surface of total NK cells and NK subsets by FCM (Fig. 3A). The TIM-3 expression on peripheral blood NK cells in SAA untreated patients (63.57 ± 12.14%) was significantly lower than that in SAA remission patients (75.88 ± 12.83%, $P < 0.05$) and normal controls (85.62 ± 9.03%, $P < 0.01$). There was no difference between SAA remission patients and normal controls (Fig. 3B). The TIM-3 expression on CD56dim NK cells in SAA untreated patients (66.41 ± 11.74%) was lower than treated patients. Thus the researchers concluded that TIM-3 expression is decreased on peripheral blood NK cells and CD56dim NK subsets in SAA untreated patients. This relates to NK cells because Tim-3 serves as a marker for NK-cell activation or maturation and when cross-linked can suppress NK cell–mediated cytotoxicity, and untreated AA results in IDA, which results in overload. However the most important thing to note for this section, is that Tim-3 serves as a marker for NK cell maturation and growth, and thus untreated patients who had IDA have less TIM-3 expression indicating a decrease in or abnormal maturation, thus a decrease in growth.

The influence of maternal stress during pregnancy on the postpartum iron status and immune maturation of infants was investigated in a nonhuman primate model. Forty infant rhesus monkeys were generated from two types of disturbed pregnancies, early or late gestation stress, and compared with 24 undisturbed controls. Prenatal stress increased the prevalence and magnitude of iron deficiency (ID) as the infants' growth-related demands for iron exceeded dietary intake from breast milk.Researchers found that differences in significant interaction that were found between prenatal condition and target type ($F4,122 = 3.18$, $p < 0.016$), which were not due to lymphocytopenia, decreased growth, or reduced NK cell numbers, as CD16+CD56+ NK cell percentages remained stable (13.9–18.9%). Thus IDA in infant rhesus monkeys from disturbed pregnancies did not result in changes in NK cell growth.

Genetically determined Hemophagocytic lymphohistiocytosis (HLH) also referred to as familial hemophagocytic lymphohistiocytosis, commonly manifest in infants or in young children and are characterized by genetic abnormalities of natural killer (NK)-cell function due to gene mutation of HO-1 (*Greil, J et al 2016)*. These abnormalities of NK cell function lead to abnormal inflammatory cytokine levels and bilirubin synthesis caused IDA (microcytic), (*Greil, J, 2016*). These findings were collected through analysis of multiple patients who have Hemophagocytic lymphohistiocytosis (HLH) and the gene mutation of HO-1. These findings of course lead to the conclusion that HLH genetic mutations of HO-1 that cause NK cell activity abnormalities lead to abnormal inflammatory cytokine levels and bilirubin synthesis causes IDA (microcytic). Inflammatory cytokines play a crucial role in the maturation of NK cells. The findings of this study relate to IDA because the conclusion that HLH genetic mutations of HO-1 that cause NK cell activity abnormalities lead to abnormal inflammatory cytokine levels and bilirubin synthesis caused IDA (microcytic) means that abnormal NK cell growth (maturation due to inflammatory cytokines) leads to IDA.

Experiments conducted in order to analyse iron, and its relation to antiviral activity in NK cells, experimented on inbred mice (*Schimmer, S et al 2025*). The experiments utilized sex- and age-matched inbred C57BL/6 mice from Harlan Laboratories, Germany, which were kept in a pathogen-free environment. The mice were at least 7 weeks

old at the beginning of the experiments. Mice were housed under 12:12 light cycle in a relative humidity of 55 ± 10 and a temperature of 22 ± 2°C. Mice were fed ad libitum with control diet (Ssniff, E15510-04, 196 mg/kg of iron) and iron-deficient chow (Ssniff, E15510-24, <10 mg iron/kg) for four weeks followed by FV infection. Rats were infected with various viruses and assessed using cell lines, infectious center assey, NK cell infection and IFn treatment, transferin uptake essay flow cyometry, Iron analysis, DFO treatment, Iron in vitro supplementation, Nk cell trasnfer/proliferation, SCENITH, and in vitro kill assey.. No significant differences in NK cell proliferation or maturation were observed. These findings suggest that increased CD71 expression is linked to iron homeostasis rather than proliferation and highlight the role of type I IFNs in regulating NK cell iron uptake. IDA increased viral replication and decreased GzmB in NK cells, suggesting iron is more crucial for immune activation than viral replication. No differences in NK cell percentages were found between untreated, DFO, or FeSO4-treated groups, but DFO reduced GzmB+ NK cells while FeSO4 increased them. Thus this study found no correlation between growth (maturation) of NK cells and IDA.

Researchers publishing a research paper assessing gene prediction of the relationship between iron deficiency anemia and immune cells *(Xu, X et al 2025*), selected IDA genetic variants, including 8376 samples and 9810691 single nucleotide polymorphisms, and immune cells from a large open genome-wide association study (GWAS) for a bidirectional MR study. The primary method was inverse variance weighting (IVW), and auxiliary analyses were MR-Egger, weighted median, simple mode and weighted mode. The reliability of the results was subsequently verified by heterogeneity and sensitivity analysis. The IVW method showed that 19 types of immune cells may be the risk factors of IDA, whereas 15 types of immune cells are the protective factors of IDA. Reverse MR analysis suggested that immune cells from upstream etiology of IDA are not involved in follow-up immune activities. Next, we selected 731 immune cell types as the results. The research revealed that IDA may result in a rise in 23 kinds of immune cells and a reduction in 12 kinds of immune cells. In addition, sensitivity analysis demonstrated no evidence of heterogeneity or horizontal pleiotropy. The paper further states that NK cells were the one shown to have a reduction in size in the case of IDA, leading to the conclusion for this study being that IDA leads to reduction in NK cell size.

C57BL/6 mice in a recent study were fed a diet consisting of 3% protein deficient in iron and zinc compared to age-matched well-nourished controls fed a 17% protein content diet supplemented with iron and zinc over 4 weeks. Circulating blood leukocytes and splenic lymphocytes were collected.Well-nourished mice had a significantly higher percentage of immature NK cells (NK1.1+CD27-CD11b-) determined by the main effect ($p=0.028$) with no impact for infection. At this early stage, the infected moderate malnourished mice showed higher early mature (NK1.1+CD27+CD11b-) cells than the other groups, with significant main effect for infection ($p=0.021$) and trending for diet ($p=0.067$) (Figure 2D). The most mature NK cell populations (NK1.1+CD27+CD11b+ and NK1.1+ CD27-CD11b+) were the same in all the groups with no significant interaction or main effects. Immature NK cells (NK1.1+CD27-CD11b-), were significantly decreased in the uninfected malnourished mice, with significant main effects for both infection ($p=0.024$) and diet ($p=0.015$) The most mature NK cell population (CD27-CD11b+) were significantly lower in the infected mice ($p=0.026$) and the well-nourished mice ($p=0.023$). Thus IDA leads to a negative impact towards NK cell maturation and growth, as IDA leads to abnormal growth in NK cells.

In conclusion, research consistently shows that iron deficiency anemia (IDA) negatively impacts the growth and function of natural killer (NK) cells. Iron deficiency leads to reduced protein synthesis, impaired NK cell proliferation, and abnormal cellular metabolism, resulting in smaller NK cells with compromised cytotoxicity and activation. Studies across different models indicate that IDA disrupts NK cell maturation, leading to abnormally grown and developed NK cells. The alteration in NK cell functionality, including decreased expression of key activation markers like TIM-3, also suggest disruptions in growth of NK cells, and have been found to do so.

Analysis of NK cell metabolism, and protein synthesis was pertinent towards reaching a conclusion as to NK cell growth, as the metabolic status and metabolic pathways of a cell are intimately tied to its ability to successfully grow, proliferate, and survive, and because protein synthesis is necessary for growth, function and metabolic pathways of a cell. Thus, decrease in context of IDA, of protein synthesis, cellular metabolism, and proteins such as TIM-3 were found to consistently correlate to decreased or abnormal NK cell growth. Furthermore 79 percent of articles agree that IDA leads to impaired or negatively impacted NK cell growth. While 21 percent of articles claim there is no correlation between NK cell growth and IDA. Moreover, 91 percent of articles that agreed with correlation claim that connections between IDA and NK cell growth are negative in the way that IDA leads to decrease or abnormality in Nk cell size. While 9.1 percent of articles argue that there is a positive correlation, as IDA leads to increased proliferation, size or health of NK cell growth. Consequently, all articles that agree with correlation, found that NK cell growth changes are caused by IDA, however one article found that Nk cell growth abnormalities can cause IDA. According to (*Greil, J et al 2016)* HLH genetic mutations of HO-1 that cause NK cell activity abnormalities lead to abnormal inflammatory cytokine levels and bilirubin synthesis that causes IDA (microcytic), which means that abnormal NK cell growth (maturation due to inflammatory cytokines) leads to IDA. While another study analysing iron and antiviral activity in NK cells of inbred mice found that iron is more crucial for immune activation than viral replication (*Schimmer, S et al 2025*). Thus, even though Nk cell growth has been found to be negatively impacted by IDA on average, proof is not concrete due to gaps in agreement and the amount of studies that disagree.

Production

Cell production refers to the creation of new cells and the increase or decrease of cell numbers, of which is usually due to mitosis or cell division. While, NK cell production refers to the creation of NK cells. NK cells are created via hematopoietic proliferating progenitor stem cells called common lymphoid progenitor cells (CLPs) that are found primarily in the bone marrow, yet are also found in the liver and thymus. Since NK cells originate from the common lymphoid progenitor, they share parentage with T and B cells because the common lymphoid progenitor (CLP) cells give rise to several types of immune cells that are part of the lymphoid lineage such as T cells, B cells, and NK cells. However, unlike T and B cells, NK cells lack immunological memory and do not undergo affinity maturation upon re-exposure to antigens, as they are part of the innate immune system and do not require thymic training.Furthermore, it is also important to consider hematopoiesis, which is the process of blood cell formation. All blood cells (red and white) originate from hematopoietic stem cells in the bone marrow. Which is why it is important to understand the distinction between progenitor cells, and precursor cells. A progenitor cell is a more committed cell that can give rise to specific types of cells, while a precursor cell is an earlier, less specialized form that has the potential to differentiate into multiple cell types before becoming fully mature. Hematopoietic progenitor cells are progenitor cells that are found primarily in the bone marrow and give rise to various blood cell types, including red blood cells, white blood cells, and platelets. There are 2 types of hematopoietic progenitor cells, Common myeloid progenitors and common lymphoid progenitors. Common myeloid progenitors give rise to innate immune cells such as (macrophages, neutrophils), and red blood cells.While Common lymphoid progenitors give rise to NK cells, B cells, and T cells.Furthermore,Nk cell production can be measured and assessed through NK cell numbers (cell count) and differentiation of NK cell maturity. NK cell count is especially important when responding to infections or tumors, because decrease in cell count results in increase in infections and tumors, and increase results in autoimmune disease. This part of the research paper will investigate changes in NK cell count in relation to IDA and stimulation. This part of the paper also aims to investigate how IDA affects NK cell creation in relation to markers like Ki-67 or BrdU incorporation and NK cell differentiation related to maturity. However, the overall guiding question for this section of the

research paper will be, How does IDA affect the ability of NK cells to produce in general, and in response to infection and stress.

Friendly Neighborhood Immunologist. (2021, September 9). *Natural Killer cells | Top 5 ways Natural Killer cells work* [Video]. YouTube. https://www.youtube.com/watch?v=aBDhnZrxAaY

Drbeen Medical Lectures. (2022, November 10). *Summary - Natural killer (NK) cells* [Video]. YouTube. https://www.youtube.com/watch?v=arz-sYRgLnk

Professor Dave Explains. (2023, December 20). *Natural killer cells: the tumor killers* [Video]. YouTube. https://www.youtube.com/watch?v=iATp8DO3RA8

A study focused on the immunological role of CD4+CD28null T lymphocytes, natural killer cells, and interferon-gamma in pediatric patients with sickle cell disease found Nk cell counts are found to be much higher in patients with SCD, and Nk cells were correlated to HbS and indirect bilirubin. To assess the percentage of CD4+CD28null T lymphocytes, natural killer cells (NK), and IFN-gamma levels, researchers compared 40 children and adolescents with SCD with 40 healthy controls and evaluated their relation to disease severity and response to therapy. Patients with SCD steady state were studied, focusing on history of frequent vaso-occlusive crisis, hydroxyurea therapy, and IFN-gamma levels. Analysis of CD4+CD28null T lymphocytes and NK cells was done by flow cytometry. Liver and cardiac iron overload were assessed. Researchers found that CD4+CD28null T lymphocytes, NK cells, and IFN-gamma levels were significantly higher in patients than controls. Patients with a history of frequent vaso-occlusive crisis and those with vascular complications had higher percentage of CD4+CD28null T lymphocytes and IFN-gamma while levels were significantly lower among hydroxyurea-treated patients. CD4+CD28null T lymphocytes were positively correlated to transfusional iron input while these cells and IFN-gamma were negatively correlated to cardiac T2* and duration of hydroxyurea therapy. NK cells were correlated to HbS and indirect bilirubin. Thus NK cell counts increase when iron is in overload.

Moreover iron is proven to affect the activation of NK cells, however the specific effects of deficiency vs surplus are yet to be highlighted (ambiguous), (*Terpiłowska, S. 2011*). These findings were developed in a literature review published in the Termedia.pl. After thorough analysis, the authors of this paper came up with the following observations A number of nutrients/ elements have the ability to modulate immune response through the production of antibodies or cytokines (e.g. zinc, selenium, chromium, iron) and may be required for immune cells proliferation or activation (e.g. iron). These elements are also required for functioning of enzymes involved in the antioxidant system (e.g. selenium) of the immune cells. It has been shown that nutrient supplementation may enhance but may also suppress immune function. Which is why the conclusion that Iron does affect proliferation/ production and activation of NK cells is accurate, however the results are quite ambiguous due to lack of evidence and lack of access to the full article.

However another study (*Rosch, L. M et al 1987*) argues that protein synthesis in the liver and thymus is not changed or affected by moderate iron deficiency. However the study did find that moderate iron deficiency did impact protein synthesis in the spleen. Protein synthesis in the spleen, liver and thymus in severely iron-deficient pups was less than half that of iron-sufficient pups, representing a severe decrease in NK cell production since protein synthesis is heavily important for NK cell production. In this study RNA, DNA and in vitro protein synthesis were measured. Rats were fed diets containing 6 (severe anemia), 11 (moderate anemia) or 250 (iron sufficient) mg iron/kg diet throughout gestation and lactation. On d 2 of lactation, litters were adjusted to contain six pups. On d 12 of lactation, two pups from each litter were immunized with sheep red blood cells (SRBC) and on d 17, tissues were removed for the determination of protein synthesis and evaluation of RNA and DNA contents.In the moderately iron-deficient pups, protein synthesis was lower (30%) in spleen than that in iron-sufficient pups. Protein synthesis in the spleen of the moderately iron-deficient group was higher after immunization with SRBC than in iron-sufficient controls, whereas the severely iron-deficient pups failed to respond. Impaired protein synthesis may be the mechanism responsible for compromised ability to produce

antibody in iron deficiency.Thus IDA leads to decrease in protein synthesis that leads to decrease in NK cell production.

Results in a research study published in the journal of immunology, indicate that thalassemia patients have a reversible, transfusion-related decrease in NK function which may arise as a consequence of iron overload (*Akbar, A. N et al 1986*). Thalassemia can be used to assess NK cell function in relation to IDA because In individuals with thalassemia, particularly those requiring regular blood transfusions, iron overload can occur due to the body's inability to effectively process the excess iron. Thus thalassemia leads to a hemochromatosis effect where it can be assumed iron is involved in and has an opposite and equal reaction to the reaction towards NK cytotoxicity expected of IDA. Researchers in this study investigated the natural killer (NK) activity of both fractionated (Percoll density gradient) and unfractionated mononuclear cells from patients with beta-thalassemia major who are iron overloaded as a consequence of chronic transfusion therapy. To determine whether or not found decrease in NK activity could be related to iron overload, researchers also preincubated patient effector cells with desferrioxamine (DFO) or 2,3-dihydroxybenzoic acid (DHB) for 6 hr before addition of K562 targets. (iron chelating agents). Both splenectomized and nonsplenectomized patients were found to have had normal proportions of Leu-11b-staining (NK) cells. Due to normal to elevated absolute white cell and lymphocyte counts, a change in the absolute number of NK cells could not account for the decreased killing even if it was observed. Proving that excess iron does not lead to abnormal production or NK cell count, and there is no correlation between iron and NK cell count.

According to a literature review completed by (*Beisel, W. 1982*) states that lymphocyte count, In vitro lymphocyte transformation,antibody production, dermal hypersensitivity, chemotaxis, Phagocytosis, bactericidal activity, and metabolic c3 are all impacted by IDA. However, analysis of multiple sources through literature review comes to the following data conclusion. Increased susceptibility to infection due to IDA via lymphoid tissues and reduced lymphocyte and NK cell counts, may lead to impaired In vitro lymphocyte transformation. While normal or impaired antibody production may have decreased delayed dermal hypersensitivity and may have increased or impaired chemotaxis, or phagocytosis impaired bactericidal activity. Metabolic c3 may increase. However no exact correlation was found between IDA and NK cell activity. Lymphocyte transformation however, is defined as the in vitro process where lymphocytes are stimulated to proliferate and change in size and appearance in response to an external threat .However, lymphocytes are a cell subtype that include an array of cells, of which are T cells, B cells and NK cells. Consequently, due to the fact that lymphocyte transformation includes multiple cells, a true conclusion cannot be made. However, if one were to assume that lymphocyte transformation refers to NK transformation and results are not altered by other cells that are a part of the lymphocyte subtype. Then the conclusion could be made that since lymphocyte transformation is affected by IDA, NK cell transformation must also be affected by IDA. Specifics on positivity or negativity towards the connection between NK cell transformation and IDA are not given in the study. However the overall conclusion of this paper is that IDA leads to reduced NK cell counts and production.

A total of 101 children who were diagnosed to have iron deficiency anemia (study group) which was related to nutritional deficiency were tested on in a 2015 research project..The control group consisted of 99 healthy children at the same ages (control group).The complete blood count (automatic cell analyzer 600), serum iron (spectrophotometry) and ferritin (RIA) were measured. A measure of 1 ml of blood sample with EDTA-containing tubes was taken by venipuncture from each patient for complete blood count including differential cell counts, hemoglobin, hematocrit, serum IgG, IgM, IgA, and ferritin levels, whole blood samples were collected. Serum immunoglobulins were measured by using commercially prepared antisera to IgG, IgA, IgM. and radial immunodiffusion.The percentage of lymphocytes with was 43,681 ± 17,936% in children with IDA and 38,199 ± 16,699% in the control group ($P<0.026$). Thus production of lymphocytes, specifically NK cells, is decreased in patients with IDA.

Twenty-two trained women runners (VO2peak 48.1 + 1.2 ml × kg-1 × min-1) were divided into an iron supplement (n = 13) or placebo group (n = 9) based on initial serum ferritin concentration (24.2 ± 2.9 and 58.5 ± 4.0 µg × l-1, respectively). Exercise consisted of a 35-min run (80 % VO2peak) and was performed at week 0 (WK0), after two weeks of intensified training (WK2) and after eight weeks recovery training (WK10). The eight weeks recovery training were concomitant with subjects taking iron supplements or placebo in a double blind fashion. Concentrations of serum ferritin, serum iron and total iron binding capacity were assessed pre-exercise and complete blood count, natural killer cell activity (NCAT), and cell surface markers for CD3+, CD4+, CD3+,CD8+, CD3-, CD16+, CD56+ cells were determined both pre- and post-exercise (Flynn et al 2007).Serum ferritin concentrations were significantly ($p < 0.05$) increased on WK10 compared to WK2 (time effect). NCAT (%lysis) and NK cell number was lower ($p < 0.05$) at WK0 for supplement (42.9 ± 1.9 % and 305.5 ± 15.0 × 106 × l-1, respectively) compared to placebo groups (50.9 ± 2.0 and 406.1 ± 25.6, respectively). Two weeks of intensified training did not alter indices of host defense. Thus the study found that NK cell numbers were lower in subjects with greater body mass and lower iron stores ($p < 0.05$), but were not significantly altered after two weeks of intensified training or when serum ferritin levels increased. Thus IDA leads to decrease in NK cell production.

Using a newly developed in vivo erythrophagocytosis assay, researchers in a new 2015 study demonstrated that activated cells of the myeloid phagocytic system display enhanced erythrophagocytosis causing acute anemia. Results from the study indicate that IFNγ plays a crucial role in the recruitment, production and activation of erythrophagocytic myeloid cells, as mice lacking the IFNγ receptor were partially protected against trypanosomosis-associated inflammation and acute anemia. NK and NKT cells were the earliest source of IFNγ during T. b. brucei infection. Later in infection, CD8+ and to a lesser extent CD4+ T cells become the main IFNγ producers. Cell depletion and transfer experiments indicated that during infection the absence of NK, NKT and CD8+ T cells, but not CD4+ T cells, resulted in a reduced anemic phenotype similar to trypanosome infected IFNγR-/- mice. Thus NK, NKT and CD8+ T cell-derived IFNγ is a critical mediator in trypanosomosis-associated pathology, driving enhanced erythrophagocytosis by myeloid phagocytic cells and the induction of acute inflammation-associated anemia. This study concludes that IDA leads to decrease in interferon gamma which is a critical mediator part of proliferation of NK cells, thus IDA leads to decrease in interferon gamma that leads to decrease in NK cell production.

Moreover a cross sectional study study on pediatric major β-thalassemia patients who routinely received a blood transfusion at Dr. Hasan Sadikin General Hospital in 2016 was conducted in order to assess hyperferritinemia and NK cell populations. Blood samples were treated with the monoclonal antibody of CD3, CD56, and CD16 to count the NK cells subsets as CD56bright, CD56dim, and CD16+ using flow cytometry. CD69+ used as an activation marker. The median fluorescence intensity (MFI) of CD56, CD16, and CD69 was measured. Total iron-binding capacity (TiBC), ferritin, and serum iron level examined as iron status. A Spearman correlation test was used for statistical analysis. Fifty-five blood samples were obtained for analysis. The study found that hyperferritinemia in pediatric major β-thalassemia patients may influence NK cell subsets' balance population, particularly the CD56bright and CD56dim NK cell subsets, then alter their immune response to pathogens. Thus iron overload is correlated to NK cell populations and productions.

A total of 64 iron-deficient patients and 19 healthy controls were included in a study at King Fahd Hospital of the University, Saudi Arabia, in order to assess IDA and its indicators on lymphocyte subsets. Complete blood counts, serum iron, ferritin, and total iron-binding capacity were assessed. Lymphocyte subsets were evaluated by flow cytometry.Among iron-deficient patients, the anemic ones (Hb ≤11 g/dL) showed significantly lower absolute lymphocyte counts ($p=0.013$), lower relative and absolute NK-cell counts ($p=0.025$ and $p=0.003$, respectively), higher relative T-cell and CD4+-cell counts ($p=0.026$ and $p=0.002$, respectively). B cells and CD8+

T cells were not affected by any iron-deficiency indicators. Iron-deficient anemia patients showed a three- to fourfold increase in risk of having recurrent infections. The study concluded that IDA has an obvious effect on lymphocyte subsets. Changes in lymphocyte subsets started mainly in response to decreased hemoglobin, rather than decreased ferritin and/or iron. Synchronously decreased hemoglobin and increased total iron-binding capacity led to absolute decreases in total lymphocytes, mainly NK cells, and relative increases in T cells, mainly the helper ones. Thus IDA and decrease in iron leads to decrease in NK cell populations and productions.

A study titled “Effects of splenectomy on natural killer cell levels in B‐Thalassemia major patients” published in the Journal of Clinical Laboratory Analysis, found that splenectomy reduces NK cell levels in patients with β-TM. The negative relationship between ferritin levels and NK cells indicates that ferritin levels should be kept under control in patients with β-TM.Seventy patients with β-TM (38 splenectomized and 32 non splenectomized) and 25 healthy controls were included in this study. The hemogram parameters, ferritin, T lymphocyte, T-helper cell, T-suppressor cell, and NK cell numbers, were measured. NK cell level was found to be significantly lower only in the splenectomy group than in the control group ($p < 0.05$). Significant negative correlations were found between serum ferritin levels and both total lymphocyte ($r = −0.617$) and CD3+ lymphocyte ($r = −0.718$) levels in the control group ($p < 0.05$). A significant negative correlation was detected between serum ferritin levels and CD3−/CD16+CD56+ NK cell levels in the patient group ($r = −0.410$) ($p < 0.05$). Thus concluding that increases in iron lead to decreases in population, leading to the conclusion that there is correlation between iron and NK cell production.

Moreover, the effect of in vitro interferon stimulation on non immune- and immune-spleen natural killer cell activity was studied in iron-deficient rat pups. Dams were fed 6, 12 or 250 mg Fe/kg diet during gestation and lactation. Approximately one-half of the 17-d-old pups were injected intraperitoneally with 105 plaque-forming units of vaccinia virus. Four d later, nonimmune and vaccinia-immune pups were killed. Spleen lymphocyte suspensions were prepared and plated with or without rat alpha/beta interferon for 2 h at 37°C. Washed lymphocytes were combined with 51 chromium-labeled YAC-1 target cells and co-cultured for 4 and 16 h at 10 and 50:1 effector-to-target ratios.Hemoglobin concentration, hematocrit, body weight, spleen weight and lymphocyte numbers per spleen were lower in iron-deficient pups than in controls. Thus leading to the conclusion that impaired spleen natural killer cell production can occur due to lack of iron in IDA of malnourished rat pups.

In another study researchers assessed how cellular metabolism relates to proliferation and effector maturation of naïve (NV) vs. cytokine-enhanced (CE) NK cells, through a literary analysis. Glycolysis was similarly induced and equally required for NV and CE NK cells to proliferate and acquire effector function. By contrast, upregulation of CD71 was a key discriminating factor between in vitro activated NV and CE NK cells, with distinctly higher cell surface expression on stimulated CE NK cells. Differential expression of CD71 translated into an increased capacity of CE NK cells to take up transferrin/iron, and was associated with higher proliferation rates. CD71-mediated iron uptake was a prerequisite for activation-induced NK cell proliferation also in vivo. In CE NK cells upregulation of the iron regulatory proteins 1 and 2 (IRP1/2) selectively created a pseudo iron deficient state. This cellular state enabled increased translation of CD71 and hence proliferation of activated CE NK cells. Thus the study found that regulating CD71 in the context of pseudo iron deficiency enabled increased proliferation of CE NK cells.

Furthermore, in a study where lymphocyte subsets and NK-cell activity were evaluated in the peripheral blood of 21 patients with iron deficiency anemia researchers determined immunological abnormalities in iron deficiency anemia which could increase the susceptibility to infections, and lower NK cell population in patients with IDA. The results of the study showed that the mean number of total lymphocytes, CD3 and CD4 subsets, and B

lymphocytes were decreased in patients of IDA. However, studies performed in 16 of these patients after the treatment revealed the recovery of these parameters except for decreased NK-cell activity. Thus IDA leads to decrease in NK cell proliferation and production.

Researchers in a 1986 study investigated the natural killer (NK) activity of both fractionated (Percoll density gradient) and unfractionated mononuclear cells from patients with beta-thalassemia major who are iron overloaded as a consequence of chronic transfusion therapy. To determine whether or not found decrease in NK activity could be related to iron overload, researchers also preincubated patient effector cells with desferrioxamine (DFO) or 2,3-dihydroxybenzoic acid (DHB) for 6 hr before addition of K562 targets. (iron chelating agents). Researchers found that due to normal to elevated absolute white cell and lymphocyte counts, a change in the absolute number of NK cells could not account for the decreased killing. Thus IDA has no impact on NK cell numbers, production or proliferation.

Moreover, a recent literature review found that total lymphocyte numbers are usually unchanged in iron deficiency, although the proportion of T cells is often reduced. The impaired T-cell function seen in iron-deficiency can therefore by explained, at least in part, by an inadequate supply of transferrin-bound iron to transferrin-receptor-bearing activated T-cells, and indeed the decreased mitogen responses of lymphocytes from iron-deficient mice were found to be due to the low saturation of serum transferrin. Total lymphocyte numbers are usually unchanged in iron deficiency, however since the decreased mitogen responses of lymphocytes from iron-deficient mice were found to be due to the low saturation of serum transferrin, It can be assumed that NK cell activity is affected by IDA especially because mitogens help activate NK cells, leading to increased proliferation, cytotoxic activity, and cytokine production. Thus IDA does not lead to any impact on NK cell production.

In conclusion, iron deficiency anemia (IDA) is consistently linked to reduced natural killer (NK) cell counts and impaired immune function. Most studies linked reduced NK cell counts and proliferation to be connected to decreased lymphocyte proliferation and protein synthesis. As when other lymphocytes were decreased due to IDA, NK cells were also decreased. Moreover, protein synthesis is necessary for all functions in all cells, including the production and creation of NK cells. Many articles analysed in this section have proven that normal protein synthesis has detrimental effects towards NK cell proliferation. Moreover, studies show that IDA can lead to increased susceptibility to infections due to lower NK cell production, proliferation and count. 81 percent of articles claim there is correlation between iron and NK cell production, while 19 percent of articles argue there is no correlation. However the exact nature of the connection remains quite ambiguous and uncertain. As 76 percent of articles argue that there is negative correlation and IDA leads to decreased NK cell proliferation. Yet 24 percent of articles claim that there is positive correlation and IDA leads to increased proliferation and benefited proliferation. In most studies NK cell counts are stated directly in relation to iron and there isn't much connection to other specific cytokines or proteins. Yet it was found that Tumor necrosis factor, and decreases found in tumor necrosis factor can prove quite influential towards recognizing IDA related decrease in NK cell proliferation. Furthermore, in a study where researchers assessed how cellular metabolism relates to proliferation and effector maturation of naïve (NV) vs. cytokine-enhanced (CE) NK cells, through a literary analysis. Researchers found that by regulating CD71, they could create a pseudo IDA that led to increased proliferation of NK cells that could be used to combat tumors. This finding is very interesting and provides many new aspects of research that could prove to hold ingenious methods of fighting cancers, tumors and infections.

Cytotoxicity

Essentially, cytotoxicity in general is a term for how toxic a substance is to cells *(News-Medical, 2021*). However the most important fact to note before delving into cytotoxicity, are the differences between Cytotoxins, Cytotoxicity, Cytokines, and Chemokines. Cytokines are small, signaling proteins produced by immune system cells that act as chemical messengers to regulate and coordinate immune responses and other biological processes. They function as the immune system's communication system and are used by immune system cells to communicate with other immune system cells (*Professional, 2025*). Cytotoxins are defined as a substance that has a toxic effect on an important cellular function; most cytokines disrupt the cellular functions that are essential to maintaining life within the cell, leading to cellular death. Chemokines are classified as groups of small, signaling proteins that act as chemoattractants who guide immune cells to specific locations in the body (*Ferrari et al., 2019*). The difference between a chemokine and cytokine is that a chemokine is a subset of cytokine that specializes in having the generic function of inducing cell migration. While the difference between a cytotoxin and cytotoxicity is that cytotoxicity is a term for how toxic a substance is to cells, while a cytotoxin is a substance that has a toxic effect on cells. In the context of an NK cell is that Cytotoxicity refers to how toxic an NK cell is to a threat, specifically it refers to the ability of NK cells to kill target cells such as virus infected or tumor cells. A cytotoxin in the context of an NK cell, is a protein released by the NK cell that kills and destroys target cells. Cytotoxic proteins are stored within secretory lysosomes, a specialized exocytic organelle found only in NK cells. Target cell recognition, which is the process of NK cells recognizing a threat, leads to the formation of a lytic immunological synapse between the NK cell and its target. Consequently, the polarized exocytosis of secretory lysosomes is then activated and these organelles release their cytotoxic contents at the lytic synapse which kill the target cell (*Topham & Hewitt, 2009*). Cytokine proteins that are the most important for an NK cell killing process are perforins and granzymes, however Fas ligand,TNF alpha and interferon gamma (IFN-Y) are all cytotoxic cytokines that are heavily involved in NK cell mediated death. When in contact with a cell the NK cell recognizes as harmful, it releases perforin that creates an opening in the target cell so the NK cell can insert granzymes that kill the cell. This part of the research paper will only assess NK cell cytotoxicity, cytotoxin behaviour and cytotoxins, in relation to IDA, and will not assess cytokines or chemokines unless they prove dysregulation in cytotoxicity.. This will be done through focusing on articles that highlight lytic granules (ex, perforin and granzymes used to induce target cell apoptosis), target cell recognition (whether IDA effects the ability of an NK cell to identify abnormal cells), and mechanisms of killing (death receptor signaling, granule exocytosis). This part of the paper will also assess IDA and its relations to interleukins (IL)-2, IL-12, IL-15, IL-18, IL-21 and type I interferons of which positively regulate NK cell function. The guiding question of this part of the paper will be, does IDA reduce NK cell cytotoxicity by affecting granule release, target cell recognition, interleukins or any other mechanism/process/ piece of data that can prove the cytotoxicity of an NK cell has been altered.

According to a study focused on assessing the effect of iron deficiency on the stimulation of natural killer cells by macrophage-produced interferon, spleen natural killer (NK) cell activity, cytotoxicity and protein synthesis are decreased in IDA (*Hallquist, N. A., et Sherman, A. R 1989*). In order to come to this conclusion, researchers took weanling male rats (n=9/group) and fed them ad libitum 5 or 37 mg iron/kg diet for eight weeks. While pair-fed rats (n=6) were fed a control diet as consumed by iron-deficient rats. Spleen NK cell activity was measured by Cr-51 release from labeled Yac-1 cells after NK cells were activated by macrophage-produced IFN. Macrophages were stimulated in vitro with poly inosinic:cytidylic acid to produce IFN. The study found that NK cell cytotoxicity after incubation with macrophage-produced IFN decreased in iron-deficient rats (13.4±1.5%) compared to control rats (19.0±1.9%). This data proves that Nk cell cytotoxicity is negatively impaired by IDA, and that NK cell cytotoxicity and ability to kill decreases in the event of an iron deficiency anemia.

A literature review completed and sourced from relevant studies published from January 1980 to January 2024 and indexed in PubMed, Google Scholar, Ovid and Scopus, with keywords iron deficiency anemia, innate and

adaptive immune system, cellular and humoral immunity, found that IDA can affect the function of natural killer cells (NK cells) and NK cell cytotoxicity because the activity and cytotoxicity of these cells increases the expression of transferrin receptors. The study also found that cytotoxicity can reduce the release of interferon gamma in viral infections. Furthermore, another study finding was that the role of iron on these innate immune cells (NK cells) is clear, especially in tumor cells *Chegni, H. et Taheri, M. S. 2024* . Knowledge of relations between IDA and tumors can be used to increase the antitumor/cytotoxic activity of natural killer cells by chelating iron (getting rid of iron), although using iron chelators such as deferoxamine (DFO) is limited due to side effects on other cells. Evidence for these claims lies in the fact that the study found that in the tumor environment (TME), natural killer cells play a role in tumor destruction by producing cytotoxic cytokines, by increasing the expression MHC1 due to the presence of inhibitory receptors, the activity of NK cells decreases, but on the contrary, when the amount of iron and ferritin heavy chain (FTH) decreases, the level of MHC1 expression also reduced and the vulnerability of cancer cells to NK cells increases. Thus IDA leads to decrease in NK cell cytotoxicity, while iron chelation in what can be assumed as hemochromatosis cases leads to increase in NK cell cytotoxicity. Leading to the final conclusion being that stable iron levels is what is necessary for proper NK cell cytotoxicity.

Moreover, another study titled " Iron deficiency anemia in children and alteration of the immune system" experimented on 200 children in order to find correlation between IDA and the immune system *Rahmani, S., et Demmouche, A 2015*.This study took in a total of 101 children for the study group, who were diagnosed to have iron deficiency anemia which was related to nutritional deficiency. While the control group consisted of 99 healthy children at the same ages. The complete blood count (automatic cell analyzer 600), serum iron (spectrophotometry) and ferritin (RIA) of all 200 children were measured. A measure of 1 ml of blood sample with EDTA-containing tubes was taken by venipuncture from each patient for complete blood count including differential cell counts, hemoglobin, hematocrit, serum IgG, IgM, IgA, and ferritin levels, whole blood samples were collected. Serum immunoglobulins were measured by using commercially prepared antisera to IgG, IgA, IgM. and radial immunodiffusion methods. Data collected lists as follows. GALAN and Al reported a reduction in the production of interleukin-2 by lymphocytes activated in iron-deficient patients. The study also found that the release of interleukin-2 is fundamental to cytotoxicity and communication between lymphocyte subpopulations and natural killer cells, but it doesn't seem to be the only cytokine which is modified by the iron status [29-31].Reported immune defects in iron deficiency include decreased cell-mediated immunity, mitogen responsiveness, cytotoxicity and natural-killer cell activity were noted. Leading to the conclusion that reported immune defects in iron deficiency include decreased cell-mediated immunity, mitogen responsiveness and natural-killer cell activity. There is also reported reduction in production of interleukin-2 cytokines that lead to decreased communication between NK cell and lymphocyte subpopulations, which lead to disruption in immune responses, cytotoxicity- potentially leading to increased susceptibility to infections and autoimmune disorders. Moreover mitogen responsiveness is essential towards cytotoxicity, and interleukin-2 is another essential cytokine towards NK cell activity. Leading to the conclusion that IDA leads to decreased NK cell cytotoxicity, due to interleukin-2, mitogen responsiveness, and cell mediated immunity.

Furthermore, according to a scoping based literature review (Badran, O et al 2024), iron plays a critical role in maintaining the cytotoxic function of NK cells. When iron levels are insufficient, the cytotoxic activity of NK cells is significantly reduced. Iron is essential for NK cells' proper activation and function. Without adequate iron, NK cells struggle to produce the necessary cytotoxic molecules, impairing their ability to eliminate tumor cells effectively. Moreover, reduction in NK cell cytotoxicity due to IDA negatively affects cancer due to the fact that NK cells are essential for controlling tumor growth. These claims were backed by the evidence that IDA impairs the production of IFN-γ, a key cytokine produced by NK cells that stimulates other immune cells and enhances tumor cell destruction .The reduced output of IFN-γ weakens the overall immune response, cytotoxicity and

further diminishes the ability of NK cells to control tumor growth, moreover the lack of ability to control tumor growth, and kill is a key marker of decreased cytotoxicity.

However, another study where the team examined immune cell phenotype and function in 21 HH patients compared to 21 healthy controls with a focus on Natural Killer (NK) cells, observed increased basal and stimulated production of pro-inflammatory cytokines such as IL-1β or IL-18 in HH patients (*Bönnemann, V 2020*). HH is a term that represents hemochromatosis, which is a disorder where extreme iron builds up- of which is of course the opposite of IDA. Thus analysing NK cell activity in relation to HH could reveal conclusions for NK cell activity in relation to IDA if it is to be assumed that IDA and HH have an equal and adverse relation to each other. The data found in this study indicates a general decrease in the total number of granulocytes in HH patients (2774 ± 958 per µl versus 3457 ± 1122 per µl in healthy controls). Demonstrating that NK cells of HH patients are not significantly affected and that the patients' treatment by regular phlebotomy is sufficient to avoid systemic iron overload and its consequences to the immune system. Leading to the conclusion that no major changes in the cytotoxic function of NK cells in HH patients were found, and that there is no correlation between IDA and NK cytotoxic function.

Researchers in another study manipulated intracellular iron levels of the human MCF-7 and MDA-MB-231 breast cancer cell lines, and measured cytolysis of breast cancer cells by the natural killer cell line NK-92MI, nitric oxide (NO) production, tumor necrosis factor alpha (TNFα) production and gene expression of ferritin heavy chain (FTH1) *Jiang, X., et Elliott, R. L. 2017.*Researchers found that NK-92MI increased synthesis and release of NO and TNFα into the medium during co-culturing of NK-92MI cells with MCF-7 or MDA-MB-231 cells. Moreover, addition of iron was found to inhibit the cytolysis of the breast cancer cell lines. While the iron chelator deferoxamine (DFOM) increased NK-92MI cytolysis to MCF-7 or MDA-MB-231 cells, and iron reversed cytotoxicity to breast cancer cells induced by NO, released from S-nitroso-N-acetyl-penicillamine (NO donor). Real time quantitative polymerase chain reaction showed that iron up-regulated the expression of FTH1 and iron chelator DFOM reduced FTH1 expression of MCF-7 and MDA-MB-231 cells. Leading to the conclusion that increased iron in cancer cells and their microenvironment protects cancer cells from natural killer cell cytolysis by antagonizing NO- and TNFα-associated cytotoxicity and by up-regulation of ferritin expression in breast cancer cells, succinctly excess iron leads to decreased cell cytotoxicity. Conversely, a decrease in iron concentration caused by DFOM improves natural killer cytolysis of tumor cells. Thus, IDA caused by DFOM improves NK cytotoxicity as cytolysis is an essential part of cytotoxicity in NK cells.

Consequently, results of a study (*Chapman, D. E.et al 1988*) assessing the in vitro natural killer (NK) activity of peripheral blood lymphocytes (PBL) in 13 patients with genetic haemochromatosis (HC) and 27 normal subjects, using a 51Cr-release cytotoxicity assay against the target K-562 leukaemia cell line, found that peripheral blood NK function and cytotoxicity is not compromised in haemochromatosis. Hemochromatosis is the excess buildup of iron in blood flow, and is the exact opposite of IDA. Thus any correlation between IDA and hemochromatosis can be used as evidence for the correlation between NK cells and IDA, assuming that IDA and hemochromatosis have an equal and adverse relation to each other. Evidence for the conclusion found by this study lies in the fact that mean NK function did not differ between the two groups of hemochromatosis patients and non patients. This conclusion differs from the reported deficit in NK activity in other diseases in which increased iron stores may occur, including alcoholic cirrhosis and β-thalassaemia major. Thus there is no relation between IDA and NK cell cytotoxicity.

In innate immunity, iron regulates macrophage polarizations, neutrophils recruitment, and NK cells activity, iron plays a pivotal role not only in the development and proliferation but also in the activation,function and most importantly, cytotoxicity of NK cells when virus infection occurs. In innate immunity, iron regulates macrophage polarizations, neutrophils recruitment, cytotoxicity and NK cells activity. Moreover, iron also plays a pivotal role

not only in the development and proliferation but also in the activation and Cytotoxicity of NK cells when virus infection occurs. Evidence for these claims are based on the following data collected by researchers conducting a literature review published in frontiers in immunology (*Ni, S 2022*) . Iron is essential for the activation of NK cells because iron inhibits differentiation and activation of Th1, Th2, Th17 and Treg cells, but it promotes CTL differentiation. Iron deficiency results in a loss function of NK cells due to negative effects on cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF) interleukin-2, IL-12, IL-15 and IL-18. vActivated NK cells increase expression of transferrin receptor (CD71) while IDA results in decrease of expression in CD71. An increased absorption of iron was found followed by NK cells activation. Furthermore, subtypes of iron-absorption NK cells are CD27+ CD11b+ NK cells. Moreover, systematic low iron levels influenced by hepcidin resulted in the suppression of NK cell activation and production of IFN-γ. Sufficient serum iron is critical to the metabolism of NK cells and their activity against virus infection. Cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF) interleukin-2, IL-12, IL-15 and IL-18 were proven to be decreased and negatively affected in relation to IDA, however the reason they are important is because these cytokines, necrosis factors and interleukins are essential towards NK cell cytotoxicity, and without them NK cells will be unable to kill properly. Thus decreases in cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF) interleukin-2, IL-12, IL-15 and IL-18, lead to the conclusion that IDA negatively impacts NK cell cytotoxicity.

Another case where thirty-four cases of people with MDS and six cases of AML transformed from MDS (MDS/AML) were studied in order to answer whether or not iron overload may promote alteration of NK cells and hematopoietic stem/progenitor cells by JNK and P38 pathway in myelodysplastic syndromes (*Hua, Y et al 2017*). In order to complete this task, HSPCs and NK cells were isolated by magnetic absorption cell sorting. Flow symmetry was used to detect the levels of ROS and intracellular JNK and P38 in NK cells and HSPCs, and total RNA and protein were extracted from NK cells and CD34+ cells to examine the expression of JNK and p38 MAPK using RT-PCR and Western blotting. The data of this study seems to indicate that intracellular iron concentration and ROS were increased in both NK cells and HSPCs in MDS patients with iron overload ($P < 0.05$). MDS patients with iron overload had higher JNK expression and lower p38 expression in NK cells, and higher p38 expression in HSPCs compared with non-iron overload groups.These results relate to IDA because Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are related bone marrow cancers, with MDS sometimes progressing to AML, and they are characterized by abnormal blood cell production and maturation.In Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukemia (AML), iron overload, often stemming from frequent blood transfusions, which is why results from this hemochromatosis diagnosis can be used in order to back up claims for IDA. The conclusion for this study was that iron overload may cause alterations and decrease in NK cell cytotoxicity and HSPCs through the JNK and p38 pathways, and play a role in the transformation to AML from MDS.

Furthermore a study focused on assessing the metabolic requirements of NK cells during the acute response against retroviral infection, (*Littwitz-Salomon et al 2021*) tested on C57BL/6 mice who were infected with 40,000 Spleen Focus-Forming Units (SFFU) of FV and had their viral loads in the bone marrow and spleens monitored over the course of 28 days. The bone marrow and spleen of these rats were analysed due to high viral replication in these organs. Since FV preferentially infects erythroblasts, monocytes and macrophages but all dividing cells in the spleen and bone marrow can be targets for infection, Iron and Nk cells were tested after the infection. This study found that IDA profoundly impaires NK cell antiviral functions, leading to increased viral loads. Moreover, Iron is very important for essential cellular activities including mitochondrial function, DNA repair and synthesis, and epigenetic regulation and sensing of hypoxia.The antiviral functions of NK cells strongly depend on sufficient levels of iron .NK cells have an increased demand for iron when responding to acute FV infection and that experimental reduction of serum iron levels inhibits NK cell activation and their IFNγ production. Smaller NK cell size and lack of killing were also observed in iron deprived cells.Evidence for these ideas can be deduced from the pieces of data. Researchers noticed an increase in the uptake of transferrin into NK cells in FV-infected

mice compared to NK cells from naive mice. Increased transferrin uptake and CD71 expression were observed in both NK cell populations from the bone marrow and the spleen following infection, suggesting that responding NK cells have an increased demand for iron.CD71 expression and transferrin uptake are mainly represented by by CD27+CD11b+ NK cells. Analysis of NK cell activation revealed a significant decrease of CD69+ NK cells in animals with reduced levels of serum iron upon acute FV infection in the spleen and bone marrow. Smaller NK cell size was also detected in the FV-infected, iron-deprived group compared to FV-infected, vehicle-treated mice. There was also a dramatic decrease of cMyc+ NK cells in mice with low serum iron upon FV infection.NK cells isolated from infected mice with low serum iron levels showed significantly less killing of FBL-3 target cells than NK cells isolated from infected mice with normal serum iron levels. Thus proving that IDA results in decrease in NK cell cytotoxicity and decrease in NK cell ability to kill.

A literature review focused on the biological and immunological aspects of iron deficiency Anemia in Cancer Development (Zohora, F. et al 2018) found that IDA changes the microenvironment of the human body by affecting both the biological and immunological systems. It increases DNA damage and genomic instability by different mechanisms. Due to IDA'S effects on the innate immune system and cytotoxicity it can be concluded that IDA leads to deterioration of NK cell cytotoxicity that leads to negative effects on the body’s cancer detection and treatment. Evidence for this claim lies in the fact that IDA interrupts the oxidative phosphorylation energy metabolism and intestinal Cytochrome-P450 systems. It also disturbs multicellular signaling pathways involved in cell survival and helps in tumor angiogenesis. Moreover, IDA is also responsible for the functional deterioration of innate and adaptive immune systems that lead to immunological dysfunctions against invading pathogens. Thus IDA is responsible for decreasing NK cell activity through disturbing multicellular signaling pathways.

In a study titled “Maternal-iron-deficiency effects on peritoneal macrophage and peritoneal natural-killer-cell cytotoxicity in rat pups” published in the American Journal of Clinical Nutrition, Cytotoxicity of peritoneal macrophages (pMs) and peritoneal natural killer (pNK) cells toward xenogenic tumor cells was studied in anemic, suckling rats. Dams were fed 6, 12, or 250 mg Fe/kg diet ad libitum throughout gestation and lactation. Pups were injected intraperitoneally with 105 plaque forming units of virus. Four days later cytotoxicity of pMs and pNK cells against YAC-1 mouse lymphoma cells was measured. Body weight, hemoglobin, hematocrit, and viable cell yield of pups were significantly decreased with decreasing dietary iron. pM cytotoxicity was significantly impaired in anemic pups at pM-target-cell ratios of 10:1 and 30:1 at 4 and 16 h ($P \leq 0.03$). pNK-cell cytotoxicity was significantly impaired in anemic pups at pNK-target-cell ratios of 10:1 and 50:1 at 16 hours. Leading to the conclusion that the Iron-deficient diet consumed by dams throughout gestation and lactation resulted in anemic offspring whose immunologic defense by pMs and pNK cells against xenogenic tumor cells was significantly reduced. Thus IDA leads to decrease in NK cytotoxicity and killer tendencies against xenogeneic tumor cells.

Pediatric major β-thalassemia patients who routinely received a blood transfusion at Dr. Hasan Sadikin General Hospital in 2016 were included in this cross-sectional study that assessed hyperferritinemia Correlated with Activated Population of Natural Killer Cells in Pediatric Major β-Thalassemia Patients (Cahyadi, A. I et al 2021). Blood samples were treated with the monoclonal antibody of CD3, CD56, and CD16 to count the NK cells subsets as CD56bright, CD56dim, and CD16+ using flow cytometry. CD69+ used as an activation marker. The median fluorescence intensity (MFI) of CD56, CD16, and CD69 was measured. Total iron-binding capacity (TiBC), ferritin, and serum iron level are examined as iron status. A Spearman correlation test was used for statistical analysis. Fifty-five blood samples were obtained for analysis. The study found that hyperferritinemia in pediatric major β-thalassemia patients may influence NK cell subsets' balance population, particularly the CD56bright and CD56dim NK cell subsets, then alter their immune response to pathogens. The fact that NK cell immune response is altered proves that cytotoxicity in NK cells decreased in thalassemia patients, proving that there is a positive correlation with NK cells and IDA.

In another study where decreased TIM-3 expression of peripheral blood natural killer cells in patients with severe aplastic anemia was analysed, (Zhang, T et al 2017) Twenty-two (twelve males, ten females) patients with a median age of 19.5 years (range 7–65) were enrolled in the study. All patients were diagnosed in the Hematology Department of General Hospital Tianjin Medical University from August 2014 to August 2015, including eleven newly diagnosed cases (six males and five females, median age of 19 years, range 9–65) and eleven remission cases (six males and five females, median age of 21 years, range 7–49) after IST.The researchers detected the TIM-3 expression on the surface of total NK cells and NK subsets by FCM (Fig. 3A). The TIM-3 expression on peripheral blood NK cells in SAA untreated patients (63.57 ± 12.14%) was significantly lower than that in SAA remission patients (75.88 ± 12.83%, $P < 0.05$) and normal controls (85.62 ± 9.03%, $P < 0.01$). There was no difference between SAA remission patients and normal controls (Fig. 3B). The TIM-3 expression on CD56dim NK cells in SAA untreated patients (66.41 ± 11.74%) was lower than treated patients. Thus leading to the study conclusion that TIM-3 expression is decreased on peripheral blood NK cells and CD56dim NK subsets in SAA untreated patients. Both of these results relate to NK cells because Tim-3 serves as a marker for NK-cell activation or maturation and when cross-linked can suppress NK cell–mediated cytotoxicity, and untreated SAA results in IDA, which results in overload.

Iron deficiency significantly reduces tumor incidence in DMBA‐treated rats by mechanisms other than NK cell cytotoxicity, TNF‐a activity, and food restriction, according to a research study where Mammary tumor incidence, natural killer (NK) cell activity, and tumor necrosis factor‐α (TNF‐α) activity were measured in iron (Fe)‐deficient and iron‐replete rats treated with the carcinogen 7,12‐dimethylbenz[a]anthracene (DMBA). Female weanling rats in the study were fed AIN‐76 diets: the iron‐deficient group was fed 5 mg Felkg diet; the control group was fed 50 mg Felkg diet; the food‐restricted group was fed 50 mg Felkg diet in the amount consumed by the iron‐deficient group; and the replete group was fed 5 mg Felkg diet for 45 days and then 50 mg Felkg diet. After six weeks of feeding, the rats were given a single intragastric dose of DMBA.Feeding the iron‐deficient diet for 20 weeks reduced hematocrit, hemoglobin, liver iron, and tumor iron values and increased spleen weight. Dietary iron repletion for 14 weeks reversed these effects of iron deficiency. Splenic NK cell cytotoxicity against YAC‐1 cells was highest in the control group. Repleting rats with 50 mg Felkg diet corrected iron deficiency but did not restore NK cell cytotoxicity. No significant differences in macrophage TNF‐α bioactivity were found among groups. Cumulative tumor incidence over all weeks was lowest in the iron‐deficient rats. Iron repletion during the promotion phase of tumorigenesis attenuates the protective effects of iron deficiency. Food restriction to the extent present in the iron‐deficient group did not protect against tumorigenesis. The iron‐deficient group had the lowest tumor burden and delayed onset of tumors. This led to the conclusion that IDA significantly reduces tumor incidence in DMBA‐treated rats by mechanisms such as NK cell cytotoxicity, TNF‐activity, and food restriction. Thus IDA leads to decrease in NK cell activity.

Results in a research study published in the journal of immunology, indicate that thalassemia patients have a reversible, transfusion-related decrease in NK function which may arise as a consequence of iron overload (*Akbar, A. N et al 1986*). Thalassemia can be used to assess NK cell function in relation to IDA because In individuals with thalassemia, particularly those requiring regular blood transfusions, iron overload can occur due to the body's inability to effectively process the excess iron. Thus thalassemia leads to a hemochromatosis effect where it can be assumed iron is involved in and has an opposite and equal reaction to the reaction towards NK cytotoxicity expected of IDA. Researchers in this study investigated the natural killer (NK) activity of both fractionated (Percoll density gradient) and unfractionated mononuclear cells from patients with beta-thalassemia major who are iron overloaded as a consequence of chronic transfusion therapy. To determine whether or not found decrease in NK activity could be related to iron overload, researchers also preincubated patient effector cells with desferrioxamine (DFO) or 2,3-dihydroxybenzoic acid (DHB) for 6 hr before addition of K562 targets. (iron chelating agents).Patients were found to have significantly decreased NK cytotoxicity against K562 targets at

all effector:target ratios tested (p less than 0.001). Moreover, the decrease in NK cytotoxicity was transfusion related (r = -0.603, p less than 0.005). When added, iron-chelating agents consistently increased the NK cytotoxicity of cells from thalassemia patients. DHB had the greater effect, being able to increase patient NK cytotoxicity to virtually normal levels. Preincubation of cells from normal controls with DHB caused only a slight increase in NK cytotoxicity, and similar treatment with DFO had little or no effect. When target cells were preincubated with the chelating agents before addition of either normal or patient effector cells, no change in cytotoxicity was seen, demonstrating that the chelating agents act at the effector cell level. If the chelating agents were saturated with iron prior to preincubation with the effectors, no increase in the cytotoxicity of thalassemic NK cells was observed. Results of this study indicate that thalassemia patients have a reversible, transfusion-related decrease in NK cytotoxicity which may arise as a consequence of iron overload.

Furthermore according to a book titled iron and the immune system, (*Brock, J. H. 2018*) total lymphocyte numbers are usually unchanged in iron deficiency, however since the decreased mitogen responses of lymphocytes from iron-deficient mice were found to be due to the low saturation of serum transferrin, It can be assumed that NK cell cytotoxicity is affected by IDA especially because mitogens help activate NK cells, leading to increased cytotoxic activity and cytotoxicity. This book is based on literature review, and is an analysis of previous works. Results of this book and conclusions related to IDA and cytotoxicity can be explained through the following pieces of data. Total lymphocyte numbers are usually unchanged in iron deficiency, although the proportion of T cells and NK cells is often reduced. The impaired T-cell and NK cell function seen in iron-deficiency can therefore by explained, at least in part, by an inadequate supply of transferrin-bound iron to transferrin-receptor-bearing activated T-cells and NK cells, and indeed the decreased mitogen responses of lymphocytes from iron-deficient mice were found to be due to the low saturation of serum transferrin. Thus the mitogen decrease indicates that NK cell cytotoxicity has been decreased through IDA.

Another study published in the *Pediatric Research journal* claims that At 4–6 mo of age, the emergence of IDA significantly accentuated an effect of prenatal stress on natural killer cell cytotoxicity. In this study the influence of maternal stress during pregnancy on the postpartum iron status and immune maturation of infants was investigated in a nonhuman primate model. Forty infant rhesus monkeys were generated from two types of disturbed pregnancies, early or late gestation stress, and compared with 24 undisturbed controls. Prenatal stress increased the prevalence and magnitude of iron deficiency (ID) as the infants' growth-related demands for iron exceeded dietary intake from breast milk. By 2 months, infants from disturbed pregnancies, especially those with ES (early stress) conditions, showed reduced NK cell cytotoxic activity against all targets ($F_{2,61} = 4.03$, $p < 0.023$). By 6 months, the difference was more pronounced, with IDA (Iron Deficiency Anemia) emerging. Cytotoxic activity against Raji and Daudi cells was further reduced in ES infants, and marginally lower against K562 cells, while controls (no IDA) showed increased killing. A significant interaction was found between prenatal condition and target type ($F_{4,122} = 3.18$, $p < 0.016$).These differences were not due to lymphocytopenia or reduced NK cell numbers, as CD16+CD56+ NK cell percentages remained stable (13.9–18.9%). Statistical modeling showed MCV correlated with reduced NK cytotoxicity, especially against Raji and Daudi cells. A greater MCV decline was associated with more significant reductions in NK activity at 6 months. Infants with lower birth weights and higher postnatal growth rates during the first two months were more likely to experience large decreases in both MCV and NK activity. Lower MCV levels were still linked to reduced NK lysis, even after accounting for growth factors, indicating that prenatal stress contributed to these changes. Nk lysis is the ability and action of an NK cell killing threats. Thus IDA is linked to decrease in NK cell cytotoxicity and NK lysis which is just NK cell killing ability in monkey infants.

In a study titled “In vitro cytokine production in patients with iron deficiency anemia” The in vitro production of interleukin (IL)-1β, IL-2, IL-6, IL-10, and tumor necrosis factor alpha (TNFα) by peripheral blood mononuclear cells (PBMC) from 20 patients with iron deficiency anemia (IDA) was examined before and after iron

supplementation and compared to values obtained for PBMC from healthy controls. Researchers through the data of the study found that a significant decrease in IL-2 production was observed in IDA patients, whereas the secretion of the other cytokines did not differ from that of controls. Moreover, addition of iron to the culture medium did not affect the secretion of IL-2 and IL-1β, but caused an increase in IL-6, IL-10, and TNF-α production. Thus concluding that removal of iron stores from the body causes a decrease in T-cell and NK cell proliferation and differentiation with subsequent cytokine secretion [1], [8], [9], [10]. The results of the present work indicate that PBMC from patients with IDA secrete less IL-2 than cells from healthy controls. This data is relative because IL-2 plays a crucial role in not only the activation and proliferation of NK cells, yet also its cytotoxicity. A reduction in IL-2 could heavily impair NK cell cytotoxicity, and decrease the NK cells killing ability as this cytokine is essential for enhancing NK cell cytotoxicity through supporting their activation. Thus IDA leads to decrease in NK cell cytotoxicity through the cytokine IL-2.

The effect of iron loading on peripheral blood lymphocyte subsets and on circulating cytokine levels in Iron-Depleted hemodialysis patients receiving erythropoietin was researched in a study published by Nephron Clinical Practice (*Tsouchnikas, I. et al 2007*). The aim of this study was to evaluate the effect of iron load on peripheral blood lymphocytes subsets and on circulating cytokine levels in HD iron depleted patients, treated with EPO. Researchers studied 19 stable adult HD patients, 12 males, with a mean age 59 ± 11 years and mean HD duration 24 ± 14 months. All patients were iron deficient and were treated with unchanged EPO dose for the last 4 months before entering the study. The administered dose of iron was infused intravenously (1,000 mg iron sucrose) in 10 doses, during 10 consecutive HD sessions. Patients were screened before the commencement of the HD session on two occasions, once prior to the first dose of iron and 2 days after the 10th dose. Hematocrit (Ht), hemoglobin (Hb), iron, serum ferritin, transferrin saturation, interleukin (IL)-2, IL-4, IL-10, interferon-γ and tumor necrosis factor-α were measured. Major lymphocyte subsets (CD3+, CD19+, CD4+, CD8+, CD16+/56+, CD3+CD16+CD56+) and the ratio CD4+/CD8+ were also determined by two-color immunofluorescent analysis using flow cytometry. The relevant data the researchers found are that Hb, transferrin saturation and ferritin increased significantly at the end of the study 11.2 ± 0.9 to 11.6 ± 0.8 g/dl, $p < 0.005$, 17.5 ± 6.9 to 23.0 ± 10.8 %, $p < 0.05$, and 70 ± 43 to 349 ± 194 μg/l, $p < 0.005$, respectively. IL-2 also increased significantly 27.8 ± 15.2 to 38.9 ± 12.8 pg/ml, $p < 0.05$. After iron load there was no significant change to the major lymphocyte subsets examined but a significant increase of the percentage and number of T lymphocytes with positive natural killer receptors (NKR T) cells was observed, 5.1 ± 3.7% to 6.3 ± 3.46%, $p < 0.05$, and 76.4 ± 40 to 101.5 ± 48 cells/μl, $p < 0.005$, respectively. Thus leading to the conclusion that iron load in iron-deficient EPO-treated HD patients did not produce any changes in major lymphocyte subsets in peripheral blood, but it resulted in a significant increase of NKR+ T cells, a subpopulation important for local immune responses. Iron load for a relatively short period improved anemia of HD patients and influenced the levels of the circulating IL-2, which may regulate factors affecting the survival of patients.An increase in IL-2 production could have a positive impact on NK cells, as IL-2 is a critical cytokine for NK cell cytotoxicity. Thus, excess iron load leads to an increase in IL-2 which is helpful towards increasing and regulating normal NK cell cytotoxicity, and increase in iron leads to positive effects on NK cell cytotoxicity. This study argues however that it is excess, over the top iron that leads to positive effects on NK cell cytotoxicity, which results in the data driven assumption that IDA would lead to decrease in NK cell cytotoxicity and negatively impact NK cell cytotoxicity.

Furthermore, another study (*Kuvibidila, S. R et al 2010*) that assessed Iron deficiency and how it reduces the secretion of interferon-gamma by mitogen-activated murine spleen cells found that Iron deficiency impairs IFN-γ secretion (cytotoxicity) by mitogen-activated murine spleen cells, IFN-γ (Interferon-gamma) plays a key role in NK cell activation. It enhances NK cell cytotoxicity by increasing the production of cytotoxic proteins like perforin and granzyme which helps and commences NK cell initiated cell death towards threats to the body. To determine an answer to the correlations between IDA and secretion of interferon gamma, and whether reduced IFN-γ contributes to impaired immunity, researchers measured IFN-γ in supernatants of activated (2.5 μg/ml

concanavalin A, 50 ng/ml anti-CD3 antibody) spleen cells from control (C), iron-deficient (ID), pair-fed (PF), and iron-replete mice for 3 (R3) and 14 days (R14) (11–12/group). Groups with the low iron (5 ppm) and control (50 ppm) diets had identical composition, except for iron composition.Mean indices of iron status after 51 days of feeding were as follows: C = PF ≈ R14 > R3 > ID ($p < 0.01$). Iron deficiency, but not pair feeding reduced IFN-γ concentration in mitogen-treated cells by 30–43% ($p < 0.05$); while iron repletion improved it. Reduced IFN-γ was not simply due to differences in IL-12 (IFN-γ inducer), percentage of CD3+ T cells, or impaired cell proliferation because these indices were not always decreased. It was likely due to a defect in T cell activation that leads to IFN-γ gene expression Moreover. IFN-γ secretion is also positively correlated with indicators of iron status, body, and thymus weights ($r = 0.238–0.472$; $p < 0.05$). Thus leading to the conclusion that IDA impairs IFN-γ secretion by mitogen-activated murine spleen cells, and that IFN-γ (Interferon-gamma) plays a key role in NK cell cytotoxicity by increasing the production of cytotoxic proteins like perforin and granzyme. Therefore IDA leads to impaired interferon gamma secretion that leads to decrease in NK cell cytotoxicity.

While another study published in the journal of animal science (*Kleinbeck, S. N., et McGlone, J. J.*1999)found many interesting results, and collected lots of data in many categories. In this study, intensive indoor versus outdoor swine production systems were assessed based on genotype and supplemental iron effects on blood hemoglobin and selected immune measures. The objectives of this experiment were to determine the effects of production system and genotype on pig performance and health and to determine whether C-15-405 pigs reared outdoors or indoors needed supplemental iron or whether they would receive enough environmental iron, and how the lack of supplemental iron may impact pig Hb and immunity. Sows were bred, gestated, farrowed, and lactated in either an intensive indoor or an intensive outdoor production system. The three dam genotypes of pigs used in each environment were PIC Camborough-15 (C-15), PIC Camborough Blue (CB), and Yorkshire × Landrace (YL). All pigs received 100 mg of iron dextran at day 3 of age. Indoor and outdoor pigs received either no supplemental iron, 100 mg, or 400 mg of iron dextran on d 3 of age. Researchers in this study found many intriguing pieces of data, such as differences in blood hemoglobin, Wbc, Genetic lines, Hb levels, and lymphocyte ratios. However, the most important piece of data regarding cytotoxicity is the finding that natural killer cell (NK) cytotoxicity was greater ($P < .05$) among indoor- than outdoor-reared pigs (NK % cytotoxicity: 15.6 ± 2.3 vs 9.7 ± 2.3). And that outdoor-reared pigs that received no injected iron had similar Hb at d 28 of age as indoor-reared pigs that received 100 mg of iron dextran (11.1 ± .36 vs 10.7 ± .4 g/dL, $P = .59$). Since pigs grown indoors had normally lower hemoglobin and diagnosed IDA, and the fact that they required iron supplements in order to not have IDA, means that the fact that NK cell cytotoxicity was greater in pigs grown inside, and less in the ones grown outside indicates that pigs with IDA had positively impacted cytotoxic activity.

Consequently, a literature review (*Aksan, A et al 2021*) focusing on IDA in colorectal cancer found that Iron deficiency disrupts the cytotoxic and specifically anti-tumor activities of NK cells and is conducive to oncogenesis and tumor growth. The study also found that release of proinflammatory cytokines that are essential for activating NK cell cytotoxicity also lead to IDA. The study supports this conclusion by providing the following evidence. Iron deficiency can limit hematopoiesis, a prerequisite for immune cell production, and iron is necessary for the correct functioning of the immune cells. The main cause of IDA in cancer is the release of cancer-associated pro-inflammatory cytokines such as interleukin (IL)-6, IL-1, TNF-α, and IFN-γ. These cytokines upregulate hepcidin synthesis, thus reducing the quantity of iron released into the circulation.Hypoxia, which is characteristic of the iron deficient state, has been shown to inhibit the expression of vital activating NK-cell receptors and NK-cell ligands on tumor cell membranes (120, 121). Iron deficiency therefore disrupts the cytotoxic and specifically anti-tumor activities of NK cells and is conducive to oncogenesis and tumor growth, while the proinflammatory cytokines that activate NK cell cytotoxicity also lead to IDA resulting in a round circle of interconnectedness.

However, another literature review (*Yadav, D., et Chandra, J.* 2010) found that iron supplementation results in higher susceptibility to malaria, of which fighting is a responsibility of the NK cell, thus the relative conclusion that can be reached from this is that there is decreased cytotoxicity in cases of over iron supplementation that leads to malaria. Evidence for this proclamation is laid out in the following segments of data. Impaired cell-mediated immunity and bactericidal function are generally noted in iron-deficient persons, however, the findings found in this literature review are inconsistent. Despite proven reversible functional immunological defects in vitro studies, a clinically important relationship between states of iron deficiency and susceptibility to infections (cytotoxicity) remains controversial. Studies from malaria endemic regions have however reported increased incidence of malaria in association with iron supplementation. Thus this study concludes that even though a relation between IDA and decreased cytotoxicity is inconsistent although, heightened iron in the body leads to increased susceptibility to malaria and decreased cytotoxic function.

A study that assessed the differential effects of iron deficiency and underfeeding on serum levels of interleukin-10, interleukin-12p40, and interferon-gamma in mice, found that iron deficiency alters the balance between pro- and anti-inflammatory cytokines, which impacts NK cell cytotoxicity because, IFN-γ enhances NK cell cytotoxicity (*Kuvibidila, S., & Warrier, R. P. 2004*). To determine whether iron deficiency alters serum levels of IFN-γ, IL-10, and IL-12 in mice, researchers measured cytokine levels by enzyme immunoassay in iron-deficient (ID), control (C), pair-fed (PF), and iron replete C57BL/6 mice for 3 (R3) and 14 (R14) days (n=24–28, 12 R14). They found that Iron deficiency was associated with ≥50% reduction in hemoglobin, hematocrit, liver iron stores, and thymus weight ($p<0.05$). Iron repletion improved these measurements. While iron deficiency significantly reduced IL-12p40 (64%) and IFN-γ (66%) levels, underfeeding reduced those of IL-10 (48%) ($p<0.05$). Iron repletion improved cytokine concentrations to PF levels. Thymus atrophy observed in 16 ID and 19 R3 mice, had no effect on IL-12p40 and IFN-γ, whereas it further decreased IL-10 levels by 72% ($p<0.05$). Cytokine levels positively correlated with indicators of iron status, body and thymus weights ($r\leq0.688$, $p<0.05$). Thus, the decrease in interferon gamma due to IDA proves a negative correlation between IDA and NK cell cytotoxicity.

In order to assess disturbances in iron homeostasis result in accelerated rejection after experimental heart transplantation, Researchers decided to identify the mechanistic influence of iron in a murine model of HTx, fully allogeneic BALB/c donor organs were transplanted into iron-overloaded or iron-deficient C57BL/6 mice, and recipients were analyzed for functional and immunological parameters. After HTx, iron overload accelerated acute rejection as observed by shortened graft survival (HTx vs HTx + iron; $p = 0.01$), elevated rejection score ($p < 0.01$), and induction of troponin T ($p < 0.01$) (Resch, T et al 2017). Compared with controls, allografts and recipient spleens derived from iron-overloaded recipients were characterized by a pronounced graft infiltration of CD4+ T cells ($p < 0.01$), CD3−NKp46+ natural killer cells ($p < 0.05$), and reduced frequencies of regulatory T cells ($p < 0.01$). This was accompanied by lower mRNA expression levels of anti-inflammatory cytokines, including interleukin-10, transforming graft factor-β, and Foxp3. ID triggered a significant influx of innate immune subsets (NK cells and DCs) as well as CD4+ and CD8+ T cells into naïve hearts. Thus the study concludes that iron overload resulted in graft infiltration of NK cells (rejection of graft, increased cytotoxicity). NK cells were also significantly induced in the graft ($p < 0.05$) and spleen ($p < 0.001$). Graft infiltration and rejection of graft occurs when NK cells attack the graft due to increased and abnormal cytotoxicity.

A series of food restriction experiments on weanling C57BL/6 mice were conducted in order to access cytokine activities and natural killer cell cytotoxicity in response to food restriction and iron deficiency in rodents (*Spear, A. T. 1992*).Weanling C57B16 mice food restricted by 20% for 5 wk exhibited conserved spleen:body weight ratio, conserved natural killer (NK) cell activity and greater interleukin-1 (IL-1) activity; while mice food restricted by 40% or 60% had reduced spleen:body weight ratio, reduced NK cell cytotoxicity and conserved IL-1

activity compared with mice fed ad libitum. Interleukin-2 (IL-2) production, interferon (IFN) a and tumor necrosis factor (TNF) a activities were not different between the carbohydrate and food restricted mice. The effect of food restriction on IL-2 production was dependent upon mitogen stimulation: basal IL-2 production was higher in the food restricted mice and mitogen-stimulated IL-2 production was lower compared with mice fed ad libitum. After 12 wk of food restriction from d 35 to d 119 of age in C57B16 mice, the NK cell response to an in vivo stimulant was impaired, but IFN a activity was conserved compared with feeding ad libitum. Twelve wk after carcinogen administration, NK cell cytotoxicity, IFN a and TNF a activities were not different between the food restricted rats and rats fed ad libitum. The study concludes that NK cell cytotoxicity appears to be negatively impacted by more severe food restriction, while moderate restriction preserves NK cell function.

Dietary and nutritional status of individuals habitually consuming a vegan diet was evaluated by biochemical, hematologic, and immunologic measures in comparison with a nonvegetarian group (Haddad, E. H et al 199). Serum ferritin concentrations were significantly lower in vegan men but iron and zinc status did not differ between the sexes. Mean serum vitamin B-12 and methylmalonic acid concentrations did not differ; however, 10 of the 25 vegans showed a vitamin B-12 deficit manifested by macrocytosis, circulating vitamin B-12 concentrations <150 pmol/L, or serum methylmalonic acid >376 nmol/L. Vegans had significantly lower leukocyte, lymphocyte, and platelet counts and lower concentrations of complement factor 3 and blood urea nitrogen but higher serum albumin concentrations. Vegans did not differ from nonvegetarians in functional immunocompetence assessed as mitogen stimulation or natural killer cell cytotoxic activity. Thus concluding that even though low iron and lymphocyte levels were observed, vegan related IDA did not result in decrease or increase in NK cell cytotoxic activity.

In a paper titled “Ironing Out the Kinks: Arming Natural Killer Cells against Ovarian Cancer”, A researcher analysed another researchers project where deferiprone, an iron chelator, was used to reduce iron in ovarian cancer cells, triggering the cGAS/STING pathway and boosting type I IFN production (Bell, H. N., et Zou, W 2024). This activated IL-15 in dendritic cells, which recruits and activates NK cells to enhance their antitumor activity. The treatment also synergized with cisplatin chemotherapy to reduce ovarian cancer metastasis and improve survival in murine models. The data taken from the study lists as the following.Iron chelator deferiprone directly reprograms ovarian cancer cells to produce increased type I IFN and enhance NK cell-mediated immunity. NK cells produced perforin and granzyme , manufacture high levels of IFNγ and TNFα, and are highly cytolytic. Type I IFN is critical for the effective activation and antitumor function of NK cells. Type I IFN induces IL15 expression by dendritic cells (DC), which then stimulates NK cell activity and recruitment. Iron chelation did not induce cytotoxicity in tumor cells alone, but rather robustly increased the number of NK cells in the peritoneal cavity. NK cell ablation using antibody therapy did not impact the survival of vehicle or cisplatin-treated mice but substantially curtailed the therapeutic impact of deferiprone. RNA sequencing analysis revealed that alongside iron-regulated genes, deferiprone repressed immunosuppressive TGFβ and IL10 while upregulating tumor cell transcripts involved in type I IFN signaling and NK-cell activation. deferiprone-induced increase in mitochondrial DNA triggers the cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS)- stimulator of IFN genes (STING) pathway, leading to the phosphorylation of IRF3 and increased expression of the type I IFN system. Iron chelation with deferiprone activates the DNA damage response in parallel through immediate phosphorylation of CHK1, IL15 can drive NK-cell proliferation and NK cytotoxicity. Type I IFN induces expression of IL15 in DCs, which in turn stimulate neighboring NK cell . Deferiprone indeed increased the proportion of tumor-associated DCs expressing high levels of IL15, and DC depletion reduced NK-cell infiltration at tumor sites. Thus concluding that iron chelation enhances type I IFN production, promotes NK cell tumor trafficking and activation, and synergizes with chemotherapy drug cisplatin to reduce metastatic ovarian cancer progression in murine models. In simpler terms removing iron promotes NK cell cytotoxicity.

Another study titled “Iron improves the antiviral activity of NK cells” conducted the following procedures (Schimmer, S et al 2025). The experiments utilized sex- and age-matched inbred C57BL/6 mice from Harlan Laboratories, Germany, which were kept in a pathogen-free environment. The mice were at least 7 weeks old at the beginning of the experiments. Mice were housed under 12:12 light cycle in a relative humidity of 55 ± 10 and a temperature of 22 ± 2°C. Mice were fed ad libitum with control diet (Ssniff, E15510-04, 196 mg/kg of iron) and iron-deficient chow (Ssniff, E15510-24, <10 mg iron/kg) for four weeks followed by FV infection. Rats were infected with various viruses and assessed using cell lines, infectious center assey, NK cell infection and IFn treatment, transferin uptake essay flow cyometry, Iron analysis, DFO treatment, Iron in vitro supplementation, Nk cell trasnfer/proliferation, SCENITH, and in vitro kill assey. After analysing findings, the study concluded that NK cells require iron to efficiently eliminate virus-infected target cells. Diet-related low iron levels lead to increased retroviral loads due to functional NK cell impairment, while iron supplementation enhances NK cell proliferation, as well as their cytotoxic efficacy. Notably, iron-treated NK cells exhibited significant metabolic changes, including mitochondrial reorganization. Interestingly, although iron supplementation decreased the NK cell’s cytokine production, it significantly improved NK cell degranulation and the expression of cytotoxicity-associated proteins. Iron deficiency impairs the function of murine and human NK cells, leading to reduced cytotoxicity and compromised immune responses.

Natural killer cell cytotoxicity, both basal and interferon gamma (IFN gamma)-stimulated, was studied in moderately and severely iron-deficient rats challenged with the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) (*Spear, A. T., et Sherman, A. R. 1992*). Female weanling rats were fed ad libitum semi purified diets containing 8, 13 or 42 mg Fe/kg. A pair-fed group was fed the 42 mg Fe/kg diet at the level consumed by the 8 mg Fe/kg group. Following 6 wk of dietary treatment, DMBA-treated rats received a single intragastric dose of DMBA. Dietary treatment was continued. Rats were killed at 1, 4, 8, 14 and 20 wk post-DMBA treatment. Natural killer cell cytotoxicity (both basal and IFN gamma-stimulated) was analyzed. Feeding the 13 mg Fe/kg diet resulted in lower NK cell activity ($P = 0.006$) and greater tumor burden ($P = 0.045$) and tumor incidence. Interferon gamma treatment relieved the lower NK cell cytotoxicity observed in moderate iron deficiency. Feeding the 8 mg Fe/kg diet impaired NK cell activity ($P = 0.006$), but tumor burden and incidence were less than in moderate iron deficiency .Thus, in conclusion iron deficiency, particularly moderate iron deficiency, contributed to cancer development and compromised NK cell cytotoxicity.

In a PHD thesis study, (*Erny 2022*) C57BL/6 mice were fed a diet consisting of 3% protein deficient in iron and zinc compared to age-matched well-nourished controls fed a 17% protein content diet supplemented with iron and zinc over 4 weeks. Circulating blood leukocytes and splenic lymphocytes were collected. Results related to cytotoxic functions are listed as so. NK cells can synthesize and release cytotoxic granules, perfin and granzyme B, to directly kill cells. Moderate malnutrition was found to elevate perforin expression by day 9 p.i. ($p=0.034$). Granzyme B expression was nearly absent in the uninfected groups while present in both infected groups ($p<0.001$, $p=0.002$). Because Moderate malnutrition increases perforin and decreases granzyme B production by NK cells at the peak of P. chabaudi infection. Thus leading to the conclusion that during P. chabaudi infection NK cell cytotoxicity was negatively impacted by IDA. As NK cells in moderate malnourished mice also produced more performance, CD11b and IFN-y. But granzyme B levels were unaffected by diet. And, moderate malnutrition increases performance and decreases granzyme B production by NK cells at the peak of P. chabaudi infection.

In conclusion, after thorough analysis of multiple papers, there appears to be a relation found between NK cell cytotoxicity and IDA. 90 percent of all articles claimed that IDA and NK cell cytotoxicity had a correlation, while 10 percent of articles claimed there was no connection between the two. Consequently, 70 percent of articles believe there is a negative correlation between IDA and NK cytotoxicity, where IDA leads to decrease in cytotoxicity. While 23 percent of articles believe there is a positive correlation, where IDA leads to increase and

benefit to NK cytotoxicity. However, increase in cytotoxicity is correlated with autoimmune phenomenons, where the body's immune system is too diligent. Increase in NK cell aggression towards NK lysis leads towards situations such as graft rejection, where donor cells are attacked by an overly diligent immune system (*Resch, T et al 2017*). NK cells induced in the graft of patients with IDA were much more likely to have the NK cells experience heightened cytotoxicity and attack the graft in overdrive. Most articles in this section agree that IDA impacts NK cytotoxicity, and that NK cytotoxicity does not impact iron levels, except for one article. According to a literature review (*Aksan, A et al 2021*) Iron deficiency disrupts the cytotoxic and specifically anti-tumor activities of NK cells and is conducive to oncogenesis and tumor growth, while the proinflammatory cytokines that activate NK cell cytotoxicity also lead to IDA resulting in a round circle of interconnectedness. Highlighting the fact that cytokines released by active NK cells can lead to IDA. Moreover, articles did not provide evidence in just numbers of tumor cells killed, data used to explain the interconnection between IDA and cytotoxicity ranged from cytokines, to interleukins to multicellular signalling pathways. Decrease in activity of cytokines that promote and activate cytotoxins such as Interferon gamma, MHCI,tumor necrosis factor alpha (TNFα), and TIM-3 can represent decrease in cell cytotoxicity. While changes in mitogen response, multicellular pathways, oxidative phosphorylation energy metabolism, intestinal Cytochrome-P450 systems and interleukins such as IL-12, IL-15 and IL-18,CD71, (IL)-1β, IL-2, IL-6, IL-10, and interleukin 2 can also shed a light on the cytotoxic status of NK cells. Although, most of all, analysis of cytotoxins such as perforin and granzymes proved to be most useful towards discerning cytotoxic status of NK cells in a system. On the contrary, MHC1, interleukin-2 and TIM-3 are also very influential. Natural killer cells play a role in tumor destruction by producing cytotoxic cytokines, by increasing the expression MHC1 due to the presence of inhibitory receptors, the activity of NK cells decreases, but on the contrary, when the amount of iron and ferritin heavy chain (FTH) decreases, the level of MHC1 expression also reduced and the vulnerability of cancer cells to NK cells increases. Moreover release of interleukin-2 is fundamental to cytotoxicity and communication between lymphocyte subpopulations and natural killer cells, and decreases are quite influential towards assessing cytotoxicity. While Tim-3 serves as a marker for NK-cell activation or maturation and when cross-linked can suppress NK cell–mediated cytotoxicity. However the most influential conclusion from this section are the implications for cancer research. Many studies found that even though decreasing iron led to decreased NK lysis, Increased iron could lead to increased NK lysis and cytotoxicity which could lead to breakthroughs in fighting cancer.

Neuroimmune interactions

In order to decipher the meaning of a " neuroimmune interaction" one must first define neuroimmunology and the term neuroimmune. Neuroimmunology is the study of how immune and neurological systems work together, and the cross regulatory impacts of their functions (*Professional, 2025*). While "neuroimmune" combines the field of neuroscience and immunology to refer to the interaction and interconnections between the nervous system and immune system, it is a word that is used to describe all the conditions and processes that connect the immune and nervous system together (*Professional, 2025*). Implicatively, the term neuroimmune interactions, refers to the bidirectional interaction, influence and communication between the neurological and immunological systems, of which is crucial for maintaining overall health, homeostasis, various physiological processes, and diseases. Specific to NK cells, neuroimmune interactions refer to the responsibilities that NK cells have towards bidirectional connections between the nervous and immune system. NK cells are important immune cells that communicate with the central nervous system (CNS), they act as the bridge's in the crosstalk between the immune system and CNS in the context of normal aging and many neurological diseases, such as CNS autoimmune diseases (i.e., multiple sclerosis), neurodegenerative diseases (i.e., Alzheimer's disease), cerebrovascular diseases (i.e., stroke), and infections. This section of the paper is highly theoretical, however it aims to look at how IDA impacts interactions between NK cells and the central nervous system, especially in the context of

neuroinflammation or neuroimmune responses. This paper will also assess IDA and its correlation with NK cells and its neurotransmitters, cytokines and other immune molecules that are involved in nervous and immune system interactions. Additionally, this paper will look at whether or not IDA can impact the brain's immune response by modulating NK cell activity. The key investigative idea in this section is, does IDA affect how NK cells interact with the brain or nervous system in terms of inflammatory responses or neuroimmune balance.

Evidence for correlations between Neuroimmune interactions, IDA, and NK cells are highly non-existent. Previous connections between IDA and the brain have been made many times, as studies have demonstrated IDA occurring during a period of brain growth spurt (<2 years age) may result in irreversible brain damage (*Yadav, D., & Chandra, J 2010*).Moreover, association of iron deficiency with febrile seizures, pica, breath holding spells, restless leg syndrome and thrombosis is increasingly being recognized, and all of the aforementioned symptoms are neural based. Moreover, Iron has been proved to be influential and necessary for protein synthesis, cytokine communication, immune cell function and neurotransmitter synthesis. Essentially, iron is necessary for most communication between cells, neurons and especially between immune cells, and the connection between IDA and NK cells is an underdeveloped yet existent branch of research. Yet this topic completely lacks conversation and any research, leading towards lost potential in a very interesting topic. The study of neuroimmune connections in relation to NK cells and IDA could pave the way for experimental treatments for autoimmune diseases, cancers and neurodegenerative diseases. Moreover NK cells have been proven to be involved in the CNS and in conversations between the neural, central nervous and immune systems. Even though there is no valid or useable data or evidence to use for a literature review on this topic at the moment, hopefully in the future this gap in research will be filled.

CONCLUSION

In conclusion, the relationship between iron deficiency anemia (IDA) and natural killer (NK) cell activity remains complex, with the majority of studies indicating a negative correlation between IDA and NK cell function. Most research suggests that IDA leads to reduced NK cell counts, impaired cytotoxicity, and abnormal NK cell growth due to decreased protein synthesis and cellular metabolism. However, a smaller proportion of studies argue that IDA may have a positive effect on NK cell activity or that the relationship is bidirectional, with both NK cell activity and IDA influencing each other. Despite discrepancies, the bulk of evidence supports the idea that IDA disrupts NK cell function, leading to increased susceptibility to infections and potentially impairing immune responses to tumors. Data collected for cell activity indicates that 87.5 percent of studies agree with correlation between cell activity and IDA while 12.5 percent disagree with correlation. Moreover, around 70 percent of articles agree there is negative correlation between NK cell activity and IDA, where IDA and decreased iron is correlated with decreased NK cell activity and not increased cell activity. While 24 percent of articles argue that there is positive correlation between IDA and cell activity, where a decrease in iron is related to an increase and positive effect on cell activity. However, 7 percent of articles resulted in conclusions where both positive and negative correlation were apparent. 82 percent of articles indicate that iron affects NK cell activity in either positive or negative manner in that specific order, while 8 percent of articles demonstrate the idea that NK cell activity is the variable that affects iron levels, and that iron levels are affected by NK cell activity and not the other way around. Additionally 10 percent of articles provide evidence for both connections, arguing that IDA impacts NK cell activity and NK cell activity impacts IDA and iron levels, and that both coexist together in a loop of interconnectedness. As for cytotoxicity, 90 percent of all articles claimed that IDA and NK cell cytotoxicity had a correlation, while 10 percent of articles claimed there was no connection between the two. Consequently, 70 percent of articles believe there is a negative correlation between IDA and NK cytotoxicity, where IDA leads to decrease in cytotoxicity. While 23 percent of articles believe there is a positive correlation, where

IDA leads to increase and benefit to NK cytotoxicity. While correlations for Neuroimmune interactions remain undetermined due to lack of evidence. While data for growth suggest that 79% of articles report that IDA negatively impacts NK cell growth, while 21% find no correlation. Among those supporting a correlation, 91% suggest that IDA decreases or alters NK cell size, while 9% argue that IDA boosts NK cell proliferation, size, or health. While production data suggest that 81 percent of articles claim there is correlation between iron and NK cell production, while 19 percent of articles argue there is no correlation. However the exact nature of the connection remains quite ambiguous and uncertain. 76 percent of articles argue that there is negative correlation and IDA leads to decreased NK cell proliferation. Yet 24 percent of articles claim that there is positive correlation and IDA leads to increased proliferation and benefited proliferation. While gaps in understanding remain, particularly regarding neuroimmune interactions and the broader implications for autoimmune diseases and cancer research, the consistent findings underscore the importance of iron in maintaining NK cell health and immune function. Further research is needed to clarify the mechanisms underlying this relationship and explore potential therapeutic strategies.