

Effects of Caffeine and Cortisol on NK cell function

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ABSTRACT

Natural Killer (NK) cells play a critical role in immune defense against cancerous and infected cells through cytotoxic activity. NK cell activity is often regulated by a variety of signalling molecules, and release of these molecules can be governed by a multitude of compounds and stimulants.

This study investigated specifically the independent and tandem effects of caffeine and cortisol on NK cell function, using an in vitro fluorescence-based cytotoxicity assay. NK cells were exposed in varying concentrations (1-10 μ M) of caffeine, cortisol, and combined caffeine-cortisol concentrations in co-culture with target cells for 48 hours.

Cytotoxicity was quantified via relative fluorescence reduction and analyzed. Results indicated that both stimulants independently reduced cytotoxicity across all concentrations tested. Caffeine exposure was relatively consistent, while cortisol exhibited dose-dependent decreases. Combined treatment resulted in lower cytotoxicity overall, but a two-way ANOVA test revealed no statistically significant interaction between the two compounds ($p=0.83$).

These findings indicate that both stimulant and stress-associated compounds can independently impair NK cell function in vitro, emphasizes the importance of considering multiple stressors when evaluating immune function, and suggest that further research is needed to explore the interactions of the two compounds under different conditions.

INTRODUCTION

Natural Killer cells¹ are a class of lymphocytes that currently make up 5-10% of all lymphocytes in the human body and play a critical role in early immune defense against infected and malignant cells (Mace, 2022). As granulocytes, NK cells hold small packages of chemicals that harm or otherwise lyse (kill) other cells, referred to as cytotoxic compounds. NK cells are capable of secreting these compounds, and more specifically, are capable of terminating human cells, similar to another type of lymphocyte, the killer

¹ Hereafter referred to as NK cells.

T cell. This ability is used in viral defense and the suppression of cancer, due to the nature of those incursions. Indeed, NK cells are often the first line of defense against these sorts of attacks, resulting in their activity classified as both innate² and adaptive³ immune responses (Sun and Lanier, 2009).

Most viral defenses involve usage of the Killer T cell, or CD8+ T lymphocyte. Classified by the surface proteins on their surface, CD8+ cells express the CD8 marker, and act similarly to NK cells. Killer T cells are produced in the bone marrow, and migrate to the thymus, where they mature and are tested on their ability to distinguish self from others. In this way, the immune system can regulate and reduce the occurrences of autoimmune disorders. However, the thymus involutes (dies out) by age 20, with peak T cell production sitting at around 14-18. (Gui et al., 2012). Due to this, adults have immense reduction in T cell production, and as the rate of new T cells significantly slows, the body relies more and more on NK cells for cancer suppression.

Regulation

The immune system is perhaps one of the most tightly regulated systems in the body. The immune system works under tight control and restraint, to avoid catastrophic incidents, involving certain activating proteins and chemicals. For a majority of lymphocytes, one of two molecules are required. MHC, or Major Histocompatibility Complex, is a type of molecule that appears on most cells. Class II MHC molecules are found in most lymphocytes, and are used to transfer an antigen from an invader to other cells, and are primarily responsible for activating killer T cells and B cells, which release antibodies.

MHC Class II is active in most adaptive responses, and is critically important. MHC Class I, however, displays internal cell stability, which certain cells like Killer T cells are able to bind to, enabling detection of cell anomalies, which is how the immune system detects infected and cancerous cells. This specialized molecule then provides a method in which an infected cell can signal to the immune system that something has indeed gone terribly wrong.

Herpes simplex virus 1 and 2, or HSV, is one of the viruses that take advantage of the previously mentioned MHC class II molecule. The virus binds to the endoplasmic reticulum⁴ of infected cells, encoding a protein that accelerates the degradation of MHC Class I (Hewitt, 2003). With this molecule gone, the infected cell becomes invisible to the immune system. HIV, or human immunodeficiency virus, is another virus that down-regulates production of MHC class I, a major factor as to why the virus is able

² Portion of the immune system that acts as first responders. Rapidly activates, nonspecific, and targets anything that isn't human. Rarely overturns an invasion on its own.

³ Portion of the immune system that usually fully repels invaders. Takes a long time to activate, but is specifically adapted (hence their name) to target a specific type of pathogen. Responsible for a majority of symptoms of illness.

⁴ Cell organelles that facilitate the production of proteins.

to incubate in infected humans for several years before either killing its host or turning chronic and developing into AIDS⁵ (Lubben et al., 2007).

The NK cell is how the immune system handles viruses that attack MHC molecules. NK cell activation is primarily governed by the “missing-self” hypothesis. This hypothesis states that NK cells detect and eliminate cells that have reduced or not present Major Histocompatibility Complex Class I (MHC-I) molecules, a molecule that all living cells with nuclei have (Kärre, 2002). By simply checking for presence of the molecule, NK cells are able to distinguish between self and other, a critical ability that is an underlying motive of the immune system. Viruses that target MHC-I molecules, then, are targeted by NK cells specifically. (Sivori et al., 2019).

NK cells do not require MHC class I to detect a cell, nor do they require MHC Class II to activate. However, NK cells rely on a balance of activating and inhibiting signals to initiate cytotoxic activity (Imširović et al., 2024). A key detail in NK cell activation is the restrictions placed upon the cell from these molecules, and as such, even subtle changes in intracellular signalling can significantly alter these cells’ function (Ramirez-Labrada et al., 2022). In requiring strict signaling to activate, NK cells are able to reduce collateral damage to the body.

The Natural Killer Cell

First discovered in the 1980s, NK cells were named after their ability to spontaneously kill tumor cells, even without pre-conditioning, unlike Killer T cells, which require specific MHC signals to recognize a cancer cell (Sun & Lanier, 2009). The ability to spontaneously kill a cell led to a refactoring of the NK cell’s classification, with further abilities such as ‘memory’⁶, a trait unique to adaptive immune cells, as well as clonal expansion, where an adaptive immune cell will proliferate rapidly from a single progenitor⁷ (Sun and Lanier, 2009). Additionally, NK cells have been shown to release an array of cytokines, critical immune signalling molecules, in a manner similar to Helper T cells⁸ (Kucuksezer et al., 2021).

It is safe to say, then, that NK cells are in a grey zone between the adaptive and innate immune response. More importantly, NK cell activation and cytotoxicity, much like the rest of the immune system, is highly sensitive to environmental and metabolic conditions. Activation of these multifaceted cells is complex, and depends on a delicate balance of signalling molecules (Kucuksezer et al., 2021). As mentioned before, cytokines enhance NK activity (Liao et al., 2013), and secretion of these cytokines can be

⁵ Acquired immunodeficiency syndrome, a disorder that involves destruction of helper T cells, and subsequently immense immunodeficiency, hence its name.

⁶ More specifically, NK cells have been shown to proliferate rapidly on contact with a previously exposed antigen, similar to memory B and T cells (Sun and Lanier, 2009).

⁷ Essentially the ‘main’ cell that all copies derive from.

⁸ Helper T cells are T cells that (not surprisingly) help the adaptive immune system in multiple ways, including activation of adaptive responses.

influenced by a wide variety of factors, including stress, caffeine, and changes in diet (Speakman et al., 2023).

Caffeine and Cortisol

Caffeine is a central nervous system stimulant widely used in energy drinks and coffee. Acting as a competitive inhibitor⁹ to adenosine, caffeine could potentially enhance immune responsiveness, since adenosine is commonly linked to suppression of immune cell activation (Fredholm et al., 1999). More specifically, caffeine intake has been associated with increases in circulating NK cells (Fletcher et al., 2011, Kantamala et al., 1990). However, chronic caffeine intake can alter cell signalling pathways involving cAMP¹⁰, which directly leads to a decrease of IL-2 (Kloubert et al., 2021). Clearly, caffeine can have an effect on NK cells, but caffeine is only one of the potential factors impacting a person's immune system.

Indeed, cortisol, a steroid hormone produced in the adrenal gland, influences metabolism, immune activity, cardiovascular activity, and the stress response (Kaur et al., 2025). Specifically, cortisol is often described as an immunosuppressant due to its negative effects on the immune system when in high concentration. (Jefferies, 1991). Interestingly, compounds such as these are not necessarily linear—acute stress has been found to actually boost immune responses, while chronic stress inhibits immune functions by altering the gene expression of immune cells (Alotiby, 2024). This spotlights the complexity of signalling molecules; it is not necessarily a given that a compound will act predictably.

Research Question and Gap

Extensive research has demonstrated independently that both caffeine and cortisol influence immune function, but few studies directly compare combined effects on cytotoxicity under controlled conditions. Additionally, because caffeine consumption tends to correlate with higher stress levels (Harley et al., 2001), these two compounds are particularly important in the discussion of immune system function. Multiple compounds may not work together strictly linearly, and so further research into the complex mechanisms between molecules and factors is warranted. With that in mind, the tandem effects of caffeine and cortisol are predicted to have negative effects on NK cell cytotoxicity, though not necessarily in a linear fashion.

⁹ Meaning it mimics adenosine's structure and prevents (inhibits) adenosine from binding to proper enzymes and activating.

¹⁰ A vital second messenger derived from ATP by adenylate cyclase, essential for intracellular signal transduction.

METHODS

Study Design

This study is designed to provide a quantifiable measure of how cortisol and caffeine interact with each other to influence NK cell activity, by directly measuring the percentage of cancer cells lysed by NK cells in vitro, also referred to as cytotoxicity. The goal of this study was to determine a correlation between caffeine and cortisol levels and NK cell cytotoxicity, and analyze the compounding effects between these two drugs.

Participants and Samples

The populations of this study were two cell lines: CD56+ (NK) cells isolated from human peripheral blood, and a HeLa tumor cell line, genetically modified to express green fluorescent protein (GFP). HeLa cells were specifically chosen due to this modification, as the natural fluorescence of the cell line simplified the research process immensely. NK cells were grown in a modified F-12 Dulbecco's Modified Eagle Medium, modified with antibiotics and antifungals in a 100:1 ratio to keep cell growth steady.

Cells were placed into a 24 well plate, seeded in units of 250 uL, roughly 250,000 cells per well, with a target goal of 1:10 tumor to NK cell ratio (mimicking cell conditions in the body). Stock solutions were prepared in concentrations of 1, 2, 5, and 10uM for both caffeine and cortisol, replacing the concentration of media with 250 uL of the chosen substances. For the tandem well, 125uL was added from both caffeine and cortisol. To prepare the solutions, 200mL of DMEM F12 was filtered through a 0.4uM filter, then added 1.14uL of 10M caffeine into 6.86mL of filtered media to produce a 8mL solution of 10uM caffeine. The solution was then split into four equal tubes, each of 10uM concentration.

From there, further dilutions were performed to reach final concentrations of 1, 2, 5, and 10uM. Tumor cells were grown in standard F12 DMEM, and incubated in a flask. The cells were added in concentrations of 75 uL per vial, to produce the desired 1:10 ratio of target to effector cells. The cell plate was prepared as diagrammed in Figure 1, with the two remaining 'empty' wells containing just cancer cells to find the maximum fluorescence.

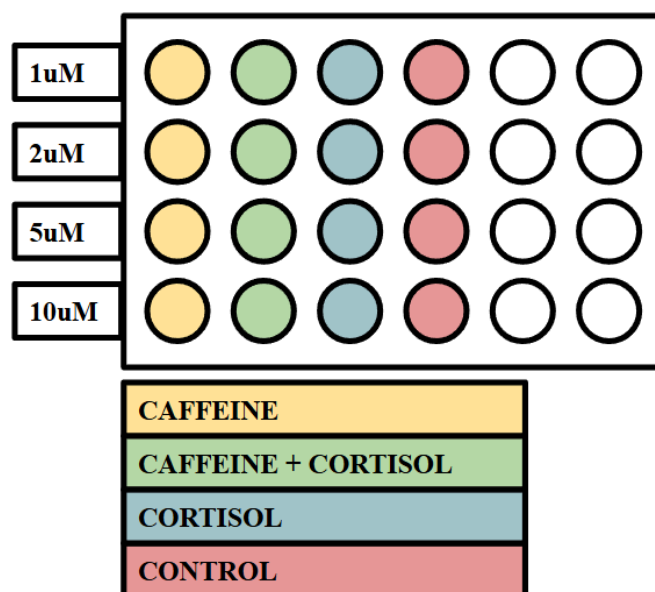


Fig 1. Cell Plate

Experiment Design

To answer the research question, there must first be a method to measure the cytotoxicity of the NK cells and if it changed due to exposure to the chosen compounds.. There were a variety of methods available. The traditional method would be a chromium-release assay, which is cited by some as “the gold standard” in terms of cytotoxicity assays (Karimi et al., 2014). The reason for this is both because of its long history of usage (dating back to 1968) and because of its rapid output (Evaluation of CAR-T cell cytotoxicity, 2022).

A chromium-release assay is a type of cytotoxicity assay that uses sodium chromate to measure the amount of target cells lysed by an effector cell. Sodium chromate, which is radioactive, binds to target cells (for this experiment, cancer cells). Once these cells are lysed by effector cells (NK cells), the rupturing of the cell membrane will release the radioactive chemical to be measured (Fehniger et al, 2014).

While highly efficient, the use of radioactive chromium is undesirable for a high school lab setting. Instead, a fluorescent GFP assay was chosen. A fluorescent assay uses the fluorescence of specific dyes¹¹ to emit color that can be measured by specific machines. While dyes would be the traditional method for fluorescent assays, the chosen HeLa cells were genetically modified to express GFP, or Green Fluorescent Protein. This way, the cells naturally and constantly fluoresce on their own, without need for any external

¹¹ Dye examples include calcein AM, SYTOX green, and propidium iodide.

dyes. Damage to the cell membrane, and subsequently cell death, would result in a diminishing of GFP fluorescence¹².

NK cells, as mentioned before, were grown in a modified F12 DMEM media, in a 24 well plate, of which sixteen wells were produced, with three rows being modified with the chosen solutions. The fourth row was filled with just NK cells with no drugs contained in them, to ensure the well environment was stable, and to ensure the cells were working as intended. Among many studies, 50-60% cytotoxicity is usually expected in healthy, functioning cells.

The DMEM used in the study, as well as cell media in general, tends to be colored a light red, due to the phenol red pH indicator contained inside it. While usually helpful to ensure the media was stable, the color can disrupt the fluorescence readings. Therefore, the plates were washed, and PBS was instead added, which maintained the cell's internal environment in a manner similar to human interstitial fluid (pH of around 7.4, low salinity, etc), while allowing the fluorescence reader to get the most accurate readings.

The plate was then placed in a Synergy HTX plate reader, and the fluorescence values of each plate were taken with excitation 480/520nm and 420/460nm. The relative fluorescence units (or RFUs) of each plate were taken and then recorded.

Fluorescence Reading

Fluorescence reading in general can be split into two halves. Part one involves the release of a photon from an external light source, provided from a lamp inside the reader. This photon excites a signalling molecule, typically hydrocarbons, known as fluorophores. An example of a fluorophore would be the green fluorescent protein involved in this experiment.

The lamp's photon strikes the fluorophore, thus exciting the molecule to a higher state of energy. The excitation values for this experiment were in the 480/520nm range. The second half involves the emission of a photon from the excited fluorophore, which returns the molecule to its ground state of energy, and releases light that can be measured. Since this photon is fundamentally weaker than the energy entering the fluorophore, the emission values were set between the 420/460 range.

Beta Testing

Preliminary beta testing was run in February 2026. Previously reviewed studies indicated usage of Dulbecco's Modified Eagle Medium as a media for cancer cells (Chava et al., 2020). However,

¹² GFP is notable for being a stable molecule, and in some cases may stay fluorescent even after cell death.

complications arising during shipment required an alternate medium be used instead. Modified F12 DMEM was chosen instead, despite not being explicitly designed for NK cells. Stock solutions were prepared as outlined in the Methods section provided above.

RESULTS

Data

Data was collected over the course of one month, between February 20th and March 26th. The actual experiment took place over the course of a week, with cell line preparation beginning on March 20th and data collection finishing on March 26th. 24 wells were prepared, including eight control wells of which the cytotoxicity % was determined, incubated at 37°C. Data was collected at a county-funded lab at X High School, with cells (HeLa and NK92MI) and basic lab equipment (filters, pipettes, etc.) provided by ThermoFisher Scientific.

The Synergy HTX plate reader was provided by Agilent, formerly BioTek. Plates were first washed with phosphate-buffered saline, to reduce fluorescent interference from the phenol red dye included in almost all media. Fluorescence was measured for each well, and each NK sample's fluorescence was divided by the average fluorescence of the cancer cell wells. Plates were run twice, with a gap of 480nm to 520nm. Relative fluorescence unit values were measured and recorded in Table 1.

Concentration	Caffeine	Caffeine + Cort	Cortisol	None
1uM	180	119	160	136
2uM	181	121	167	130
5uM	179	132	176	140
10uM	177	169	189	131

Table 1: Fluorescence intensity (RFU)

These values were divided by the maximal fluorescence measured from cancer cells, resulting in fluorescence percentages. From these percentages, the cytotoxicity could be determined, as detailed in Table 2 and Figure 2.

	Caffeine	Caffeine + Cort	Cortisol	None
1uM	35.86%	24.43%	42.83%	50.87%
2uM	36.76%	23.72%	40.87%	53.19%
5uM	35.33%	22.82%	38.72%	49.98%
10uM	36.04%	21.75%	32.11%	53.01%

Table 2: Cytotoxicity of samples (%)

Cytotoxicity (%) vs Concentration & Compound

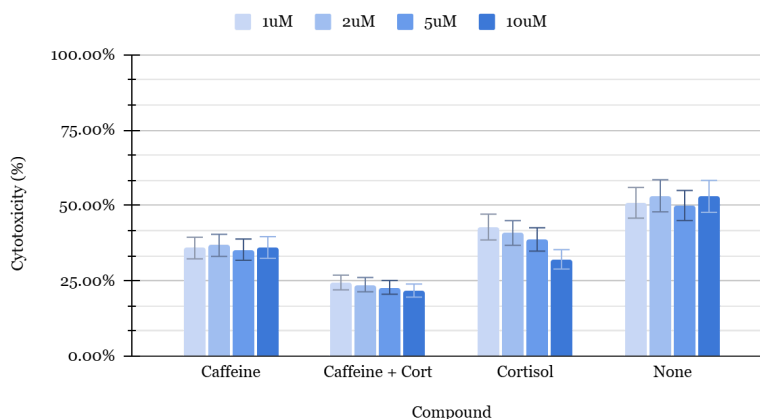


Fig 2. Graph of Cytotoxicity vs Concentration & Compound

DISCUSSION

Across all tested concentrations (1 μM –10 μM), caffeine exposure resulted in a consistent reduction in NK cell cytotoxicity, with values ranging from approximately 35–37%, compared to untreated controls, which ranged from ~50–53%. This represents an average decrease of approximately 15–20% in cytotoxic activity, indicating a substantial suppressive effect of caffeine on NK cell function. Untreated controls aligned with previous studies in the field, as ~50–60% is a standard percentage of cytotoxicity (Dons'koi et al., 2020). Cortisol exposure also resulted in a dose-dependent decrease in cytotoxicity, with values declining from ~42.83% at 1 μM to ~32.11% at 10 μM .

This trend suggests that higher cortisol concentrations are associated with progressively greater immunosuppression. A two-way ANOVA test was conducted to evaluate the effects of both compounds on NK cell cytotoxicity. Significant main effects were observed for both caffeine exposure ($p < 0.001$) and cortisol exposure ($p < 0.001$), indicating that both compounds independently reduced NK cell cytotoxicity. However, the test revealed no statistically significant interaction effect between the two compounds ($p = 0.83$), suggesting that the tandem effects may not differ significantly from additive suppression under tested conditions.

Thus, to answer the question of “what are the effects of caffeine and cortisol on NK cell activity”, caffeine and cortisol have significant negative impacts on NK cell activity, while the two compounds in tandem have an unknown relationship with one another. This finding suggests further research into tandem compounds to find other relations or perhaps deeper connections between these two compounds.

CONCLUSION

Implications

This study investigated the effects of caffeine and cortisol, both independently and in combination, on NK cell cytotoxicity in vitro. Since statistical analysis found no significant interactive effect, the tandem effects of compounds may be more subtle than initially hypothesized. The relationship between these two compounds suggests future immunological research should consider combined environmental and metabolic stressors, rather than just one single compound in isolation. Researchers studying similar behavior in NK cells may benefit from incorporating multiple factors that directly reflect real-world conditions.

As for the public sector, these findings may contribute to broader public awareness of how stress and stimulant consumption can interact in complex ways, and further investigation may be warranted in high-stress occupations with higher stimulation consumption or contexts in which immune function is already comprised (i.e the elderly population). However, because this study was an in vitro study and used isolated NK cells, further in vivo and clinical studies are necessary before making occupational recommendations.

Limitations

The proportion of my experiments likely contributes to some error in data collection. Ideally, the entire process would have been conducted over a week, rather than a month. Additionally, while the cell plate was run multiple times, more plates and thus more cultures would have ensured more accurate results. Due to a blizzard, delivery of the cells was pushed back, and the experiment had to be split into two parts—preparing the NK cells took place before the blizzard, and the HeLa cells (the delivery of which were delayed by the storm) took place after.

This delay, which lasted over a week, may have resulted in some NK cells dying before the experiment was begun, which might have led to some inconsistent results. Finally, these results cannot be interpreted as a cause for major change. Further research should expand the aims of this study to a bigger population and drug samples, and possibly consider in vivo studies, to further quantify the tandem effects of other molecules.

Future Research

Future research may want to expand upon my original method. For example, clinical studies, while challenging to track down patients, will provide more realistic data, especially since caffeine usually creates significant alterations in bodily function—such as competitively inhibiting adenosine and ADH (antidiuretic hormone).

For in vitro studies, researchers may want to avoid GFP expressing HeLa cells—even though the GFP expression is useful and efficient, GFP is an exceptionally stable protein, and while it does denature after cell death, in many cases the protein continues fluorescing even after cell death. Additionally, chromium-release assays are often more accurate and effective, and as such a research team able to handle radioactive materials should use a CRA, rather than the assays detailed in this procedure. Alternatively, research teams could consider cell-counting instead, such as flow cytometry outlined by Somanchi et al., which may result in more accurate results, if not as automated.

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REFERENCES

- Alotiby, A. (2024). Immunology of stress: A review article. *Journal of Clinical Medicine*, 13(21), 6394. <https://doi.org/10.3390/jcm13216394>
- Amling, L., Rink, L., & Bennstein, S. B. (2025). Short-term oral zinc supplementation enhances natural killer cell functionality and decreases circulating innate lymphoid cell counts and frequencies in healthy young adults. *Journal of Translational Medicine*, 23(1). <https://doi.org/10.1186/s12967-025-06259-y>
- Baddam, S., Maxfield, L., Shukla, S., & Crane, J. S. (2025). Zinc Deficiency. In *StatPearls*. StatPearls Publishing.
- Chava, S., Bugide, S., Gupta, R., & Wajapeyee, N. (2020). Measurement of natural killer cell-mediated cytotoxicity and migration in the context of hepatic tumor cells. *Journal of Visualized Experiments*, (156). <https://doi.org/10.3791/60714>
- Dons'koi, B. V., P. Chernyshov, V., Osypchuk, D. V., Sudoma, I. O., G. Khazhlyenko, K., Strelko, G. V., & Sirenko, W. J. (2020). Natural killer frequency determines natural killer cytotoxicity directly in accentuated zones and indirectly in "moderate-to-normal frequency" segment. *Central European Journal of Immunology*, 45(3), 315-324. <https://doi.org/10.5114/ceji.2020.101263>
- Evaluation of car-t cell cytotoxicity: Real-time impedance-based analysis. (2022). *Methods in Cell Biology*, 81-98. <https://doi.org/10.1016/bs.mcb.2021.08.002>

- Fehniger, T. A., Bluman, E. M., Porter, M. M., Mrózek, E., Cooper, M. A., VanDeusen, J. B., Frankel, S. R., Stock, W., & Caligiuri, M. A. (2000). Potential mechanisms of human natural killer cell expansion in vivo during low-dose il-2 therapy. *Journal of Clinical Investigation*, 106(1), 117-124. <https://doi.org/10.1172/jci6218>
- Fletcher, D. K., & Bishop, N. C. (2011). Effect of a high and low dose of caffeine on antigen-stimulated activation of human natural killer cells after prolonged cycling. *International Journal of Sport Nutrition and Exercise Metabolism*, 21(2), 155-165. <https://doi.org/10.1123/ijnsnem.21.2.155>
- Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A., & Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological reviews*, 51(1), 83–133.
- Grudzien, M., & Rapak, A. (2018). Effect of natural compounds on NK cell activation. *Journal of Immunology Research*, 2018, 1-11. <https://doi.org/10.1155/2018/4868417>
- Gui, J., Mustachio, L. M., Su, D. M., & Craig, R. W. (2012). Thymus Size and Age-related Thymic Involution: Early Programming, Sexual Dimorphism, Progenitors and Stroma. *Aging and disease*, 3(3), 280–290.
- Hartley, T. R., Lovallo, W. R., Whitsett, T. L., Sung, B. H., & Wilson, M. F. (2001). Caffeine and stress: Implications for risk, assessment, and management of hypertension. *The Journal of Clinical Hypertension*, 3(6), 354-382. <https://doi.org/10.1111/j.1524-6175.2001.00478.x>
- Hewitt, E. W. (2003). The MHC class I antigen presentation pathway: Strategies for viral immune evasion. *Immunology*, 110(2), 163-169. <https://doi.org/10.1046/j.1365-2567.2003.01738.x>
- Imširović, V., Wensveen, F. M., Polić, B., & Jelenčić, V. (2024). Maintaining the balance: Regulation of Natural Killer cell activity. *Cells*, 13(17), 1464. <https://doi.org/10.3390/cells13171464>
- International Food Information Council. (2024, March). *IFIC spotlight survey: CAFFEINE*. https://ific.org/wp-content/uploads/2025/04/IFIC-Spotlight-Survey_Caffeine.pdf
- Jefferies, W. (1991). Cortisol and immunity. *Medical Hypotheses*, 34(3), 198-208. [https://doi.org/10.1016/0306-9877\(91\)90212-h](https://doi.org/10.1016/0306-9877(91)90212-h)
- Kantamala, D., Vongsakul, M., & Satayavivad, J. (1990). The in vivo and in vitro effects of caffeine on rat immune cells activities: B, T and NK cells. *Asian Pacific journal of allergy and immunology*, 8(2), 77–82.
- Karimi, M. A., Lee, E., Bachmann, M. H., Salicioni, A. M., Behrens, E. M., Kambayashi, T., & Baldwin, C. L. (2014). Measuring cytotoxicity by bioluminescence imaging outperforms the standard chromium-51 release assay. *PLoS ONE*, 9(2), e89357. <https://doi.org/10.1371/journal.pone.0089357>
- Kärre, K. (2002). NK cells, MHC class I molecules and the missing self. *Scandinavian Journal of Immunology*, 55(3), 221-228. <https://doi.org/10.1046/j.1365-3083.2002.01053.x>
- Kaur, J., Gandhi, J., & Sharma, S. (2025). Physiology, cortisol. *StatPearls*.
- Kloubert, V., Wessels, I., Wolf, J., Blaabjerg, K., Janssens, V., Hapala, J., Wagner, W., & Rink, L. (2021). Zinc deficiency leads to reduced interleukin-2 production by active gene silencing due to enhanced crema expression in T cells. *Clinical Nutrition*, 40(5), 3263-3278. <https://doi.org/10.1016/j.clnu.2020.10.052>

- Kogevinas, M., Sala, M., Boffetta, P., Kazerouni, N., Kromhout, H., & Hoar-Zahm, S. (1998). Cancer risk in the rubber industry: A review of the recent epidemiological evidence. *Occupational and Environmental Medicine*, 55(1), 1-12. <https://doi.org/10.1136/oem.55.1.1>
- Kucuksezer, U. C., Aktas Cetin, E., Esen, F., Tahrali, I., Akdeniz, N., Gelmez, M. Y., & Deniz, G. (2021). The role of natural killer cells in autoimmune diseases. *Frontiers in Immunology*, 12. <https://doi.org/10.3389/fimmu.2021.622306>
- Lee, D. J., Ahn, S., McClure, L. A., Caban-Martinez, A. J., Kobetz, E. N., Ukani, H., Boga, D. J., Hernandez, D., & Pinheiro, P. S. (2023). Cancer risk and mortality among firefighters: A meta-analytic review. *Frontiers in Oncology*, 13. <https://doi.org/10.3389/fonc.2023.1130754>
- Liao, W., Lin, J. X., & Leonard, W. J. (2013). Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. *Immunity*, 38(1), 13-25. <https://doi.org/10.1016/j.immuni.2013.01.004>
- Lubben, N. B., Sahlender, D. A., Motley, A. M., Lehner, P. J., Benaroch, P., & Robinson, M. S. (2007). HIV-1 nef-induced down-regulation of MHC class I requires ap-1 and clathrin but not pacs-1 and is impeded by ap-2. *Molecular Biology of the Cell*, 18(9), 3351-3365. <https://doi.org/10.1091/mbc.e07-03-0218>
- Mace, E. M. (2023). Human natural killer cells: Form, function, and development. *Journal of Allergy and Clinical Immunology*, 151(2), 371-385. <https://doi.org/10.1016/j.jaci.2022.09.022>
- Mitchell, D. C., Knight, C. A., Hockenberry, J., Teplansky, R., & Hartman, T. J. (2014). Beverage caffeine intakes in the U.S. *Food and Chemical Toxicology*, 63, 136-142. <https://doi.org/10.1016/j.fct.2013.10.042>
- Oo, A. M., Mohd Adnan, L. H., Nor, N. M., Simbak, N., Ahmad, N. Z., & Lwin, O. M. (2020). Immunomodulatory effects of flavonoids: An experimental study on natural-killer-cell-mediated cytotoxicity against lung cancer and cytotoxic granule secretion profile. *Proceedings of Singapore Healthcare*, 30(4), 279-285. <https://doi.org/10.1177/2010105820979006>
- Palmer, D. B. (2013). The effect of age on thymic function. *Frontiers in Immunology*, 4. <https://doi.org/10.3389/fimmu.2013.00316>
- Ramírez-Labrada, A., Pesini, C., Santiago, L., Hidalgo, S., Calvo-Pérez, A., Oñate, C., Andrés-Tovar, A., Garzón-Tituaña, M., Uranga-Murillo, I., Arias, M. A., Galvez, E. M., & Pardo, J. (2022). All about (NK cell-mediated) death in two acts and an unexpected encore: Initiation, execution and activation of adaptive immunity. *Frontiers in Immunology*, 13. <https://doi.org/10.3389/fimmu.2022.896228>
- Shabsoug, B., Khalil, R., & Abuharfeil, N. (2008). Enhancement of natural killer cell activity in vitro against human tumor cells by some plants from Jordan. *Journal of Immunotoxicology*, 5(3), 279-285. <https://doi.org/10.1080/15376510802312027>
- Sivori, S., Vacca, P., Del Zotto, G., Munari, E., Mingari, M. C., & Moretta, L. (2019). Human NK cells: Surface receptors, inhibitory checkpoints, and translational applications. *Cellular & Molecular Immunology*, 16(5), 430-441. <https://doi.org/10.1038/s41423-019-0206-4>
- Somanchi, S. S., McCulley, K. J., Somanchi, A., Chan, L. L., & Lee, D. A. (2015). A novel method for assessment of natural killer cell cytotoxicity using image cytometry. *PLOS ONE*, 10(10), e0141074. <https://doi.org/10.1371/journal.pone.0141074>

- Speakman, S., White, K., LaPorta, A. J., Payton, M. E., Gubler, K. D., & Ryznar, R. J. (2023). Cytokine fluctuation during acute stress is correlated to life trauma. *Journal of Trauma and Acute Care Surgery*, 95(4), 535-541. <https://doi.org/10.1097/ta.0000000000004006>
- Weaver, D. F. (2022). Alzheimer's disease as an innate autoimmune disease (AD): A new molecular paradigm. *Alzheimer's & Dementia*, 19(3), 1086-1098. <https://doi.org/10.1002/alz.12789>