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# Caseins as Regulators of Hematopoiesis

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## Abstract

The main physiological role of casein, the main protein component in the milk, is to be a source of amino acids that are required for the growth of the neonate; therefore, casein is considered a highly nutritious protein. Over time, it has been revealed that casein is a protein whose physiological importance reaches levels far superior to the food field, having a wide array of biological activities including antimicrobial activities, facilitating absorption of nutrients, as well as acting as a growth factor and an immune stimulant. Here we analyze how caseins can exert numerous hematopoietic and immunomodulatory actions, their role in granulopoiesis, monocytopoiesis, and lymphopoiesis from the early stages of postnatal development seemingly throughout life, and we wonder if casein could be useful to fight pathogens resistant to antibiotics, inducing a strong immune response in immunosuppressed patients, or even be a prophylactic strategy to prevent infections.

**Keywords:** granulopoiesis, monocytopoiesis, lymphopoiesis, milk proteins, sodium caseinate

## 1. Introduction

Hematopoiesis is a process that includes the formation, maturation, and differentiation of blood cells. These cells have a relatively short life in circulation, so blood is a tissue with a high rate of renewal. The production of hematopoietic cells depends on a highly specialized bone marrow microenvironment, which regulates the quiescence, differentiation, and self-renewal of a rare population of multipotent cells known as hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs), which give rise to all hematopoietic cell lineages [1].

The hematopoietic system plays numerous essential roles in human health and disease. Failure to maintain homeostasis in the blood system results in a range of human diseases, including anemia, hemophilia, immunodeficiency, allergies, leukemia, and lymphoma [2].

Studies in the past have consistently demonstrated that diet and nutritional status can significantly alter organismal physiology [3]. Thus, Kornberg et al. demonstrated over 60 years ago that amino acids were required for granulocyte and erythrocyte production; now, it is evident that disruption of dietary and metabolic factors [4], such as inadequate or imbalanced intake of macronutrients (carbohydrates, proteins, and fats) and micronutrients (vitamins and minerals), also known as “malnutrition,” alters hematopoiesis and is generally associated with health risk

markers [3]. In this sense, the organismal diet has emerged as an important regulator of adult HSC function [5].

Of the different types of malnutrition, protein restriction results from insufficient protein ingestion that can modify physiological responses and induce cellular disturbances, especially in tissues with a high rate of cellular renewal and proliferation, such as hematopoietic tissue; such process requires a large supply of nutrients as well as an organized structure for proliferation [6]. Protein malnutrition causes modifications to the blood tissue, hampering the development and maturation of hematopoietic cells, and these changes could be the cause of anemia, leukopenia, and bone marrow hypoplasia [1].

Protein malnutrition can disrupt numerous processes in hematopoiesis, causing damage to the hematopoietic niche, stromal cells, and the extracellular matrix, and they can result in cell death in bone marrow [1]. However, these issues are not only a consequence of inadequate nutrient supply. Here, we analyze how protein intake, in particular, caseins, the main proteinaceous component of milk, can exert numerous hematopoietic and immunomodulatory actions in addition to performing their nutritional properties [7] from the early stages of postnatal development seemingly throughout life.

## 2. Casein

Milk proteins can be broadly classified into three categories: caseins, whey proteins, and mucins [8], as proteins present in the milk fat globule membrane. In milk, caseins interact with calcium phosphate, forming large stable colloidal particles termed micelles. The micelles consist of casein molecules, calcium, inorganic phosphate, and citrate ions [9]. These micelles make it possible to maintain a supersaturated calcium phosphate concentration in milk, providing the newborn with sufficient calcium phosphate for the mineralization of calcified tissues [10].

Casein (from the Latin word *caseus* for cheese) comprises the major protein component of milk of most mammals [11], but relative proportions of caseins differ widely between species. In this sense, caseins comprise approximately 80% of the total protein in ruminant milk [12], but only about 55% of the total protein in horse milk [13].

Casein, which is a phosphoprotein, contains 0.7–0.9% phosphorus that is covalently bound to the protein by serine ester linkages [9], composed of many hundreds of individual amino acids, each of which may have a positive or a negative charge depending on the pH of the milk system. All amino acids that are essential to humans are present in casein in high proportions, with the possible exception of cysteine [9]. It is widely accepted that the main physiological role of casein in the milk system is to be a source of amino acids that are required for the growth of the neonate; therefore, casein is considered a highly nutritious protein. However, the dominant physiological role of the casein micelle system is to prevent pathological calcification of the mammary gland [14]. Over time, it has been revealed that casein is a protein whose physiological importance reaches levels far superior to the food field, having a wide array of biological activities including antimicrobial activities, facilitating absorption of nutrients, as well as acting as a growth factor and an immune stimulant [15].

Caseins are consist of at least three and normally four gene products and further divided into  $\alpha$ S1-casein,  $\alpha$ S2-casein,  $\beta$ -casein, and  $\kappa$ -casein in farm animals [11, 16] and human, and each has slightly different properties that are caused by small variations in their amino acid content. The four different types are known to occur in at least 10 genetic variants (A1–A3 and B–H) from which the A2, A1, and B forms are the most prevalent [9].

Casein is the major component of bovine milk, whereas whey is predominant in human milk. The human milk whey/casein ratio changes over the course of lactation, declining from 90/10 in colostrum (days [d] 0–5) to 65/35 in transitional milk (d6–15), then 60/40 beginning at 1 month postpartum, and continuing throughout the first year of lactation [8].

Caseins are synthesized in the mammary gland and are under multihormonal control, and in the bovine genome, they are linked within a 200-kb region on chromosome 6, in the order  $\alpha$ S1-,  $\beta$ -,  $\alpha$ S2- and  $\kappa$ -casein [17].

Bovine milk caseins are composed mainly of equal amounts of  $\beta$ -casein and  $\alpha$ S1-casein [11] also contains  $\kappa$ -caseins [18], whereas human milk contains  $\beta$ - and  $\kappa$ -casein and a low concentration of  $\alpha$ -casein. The whey/casein ratio in the formula is similar to that of mature human milk (60/40), but the formula contains all bovine milk caseins. The concentrations of total caseins and  $\beta$ - and  $\kappa$ -casein increase slightly between early and transitional milk before declining and remaining relatively stable in mature milk. In contrast, the concentration of  $\alpha$ -casein is constant throughout lactation [19].

$\alpha$ S2-casein is the most calcium-sensitive member of the casein family; the sensitivity is potentially due to its high ester phosphate content, which ranges from 10 to 13 phosphate groups per peptide chain [20].  $\alpha$ S2-casein comprises up to 10% of the casein fraction in bovine milk; it consists of two major and several minor components that exhibit varying levels of posttranslational phosphorylation [21] as well as minor degrees of intermolecular disulfide bonding [22].  $\alpha$ S1-casein is only found in trace amounts in human milk (between 3 and 540  $\mu$ g/mL postpartum) [23] and is thus unlikely to function as a significant amino acid source for breastfed infants [24].

$\beta$ -Casein has 209 amino acids. The presence of proline or histidine at the 67th position of  $\beta$ -casein allows the distinction between two types of milk, A1 and A2; otherwise, there are no other differences between the two caseins. A1  $\beta$ -casein is a major variant of  $\beta$ -casein in the milk of the common dairy cows of north European origin: Friesian, Ayrshire, British Shorthorn, and Holstein. A2  $\beta$ -casein is predominantly found in the milk of Channel Island cows, Guernsey and Jersey cows, Southern French breeds, Charolais and Limousin cows [25], and Zebu original cattle of Africa. The presence of proline or histidine at the 67th position of  $\beta$ -casein is associated with a major effect in terms of bioactive peptide release by different gastrointestinal enzymes [26]; thus, a bioactive seven-amino-acid peptide,  $\beta$ -casomorphin-7 (BCM-7), can be more easily released in the small intestine by digestion of A1  $\beta$ -casein with pepsin, leucine aminopeptidase, and elastase, but the alternative proline at position 67 prevents a cleavage at this site [27].

$\kappa$ -Casein contains only one cysteine residue [28, 29], which implies that it is unable to form homomultimers, but it is capable of making one intermolecular disulfide bond [10]. In bovine milk,  $\kappa$ -casein exists as homomultimers cross-linked by random disulfide bonds [22], and it plays a key role in maintaining the stability and solubility of the micelle. Thus, the other caseins do not seem to have a role that requires well-defined structures, and  $\kappa$ -casein may well be more structured to fulfill its function as the interface between the calcium-sensitive caseins and milk serum [30]. In that role,  $\kappa$ -casein naturally resides at the surface of the casein micelle [31].

### 3. Casein and hematopoietic tissue

Low protein intake can affect all systems and organs, but it primarily affects tissues with a high rate of cell turnover, such as hematopoietic tissue [6]. Recently, Hastreiter et al. [32] compared a low-protein diet based on 20 g/kg casein with a control diet based on 120 g/kg casein, and they showed that male C57BL/6 mice after the period of malnutrition presented with peripheral leukopenia and a



reduction in lymphocytes and monocytes, especially in granulocytic cells associated with bone marrow hypoplasia. Therefore, hematopoietic stem cell (Lin-Sca-1+c-Kit+-LSK) and progenitor cell (CD45+CD34+) populations were decreased in malnourished animals [33], but also low protein intake induced a specific reduction in granulocyte-monocyte progenitors (Lin-IL7r-c-Kit+Sca-1-CD16/32<sup>high</sup>), which explains, in part, why there was a reduction in mature granulocytes [32].

It is well known that in protein malnutrition states, the number of granulocytic cells, especially neutrophils, is reduced, which predisposes patients to higher susceptibility to infection [34, 35]. However, this involvement in hematopoiesis cannot be explained only because there are not enough amino acids to support the requirements of an expanding tissue; other cellular mechanisms are involved. In this sense, Hastreiter et al. showed that there is an impaired ability of c-Kit+ cells from the bone marrow of malnourished animals to produce CFU-GEMM and CFU-GM cells, which are myeloid progenitors and, consequently, are the cells responsible for granulopoiesis [36]; this malfunction is related to Kit+ cells exhibiting reduced expression of the receptor of granulocyte colony-stimulating factor (G-CSFr), which is a granulopoietic cytokine [32].

Interestingly, Domínguez-Melendez et al. showed that administration of casein as sodium caseinate in BALB/c mice increased the percentage of myeloid precursors from bone marrow and increased the total number of bone marrow leukocytes resulting from cell proliferation. They also found that casein induced proliferation and activation of granulocytes, and it increased the serum concentration of cytokines such as G-CSF, M-CSF, and GM-CSF [37]. These cytokines are key to the proliferation, differentiation, and activation of granulomonocytic cells, which in turn induce multiple functions within the immune response; since these cytokines regulate inflammation, as is the case for monocyte-macrophages, they are responsible for phagocytosis, which is a crucial event in fungal and bacterial infections; once activated, macrophages are the bridge between activated CD4+ lymphocytes and the adaptive immune response [38]. For the lymphoid lineage in vivo, sodium caseinate also influences the induction of IL-7, a key cytokine involved in lymphopoiesis of B cells, which are key cells for the adaptive immune response, since once activated, they are responsible for producing antibodies that they will opsonize foreign antigens to facilitate their elimination [39].

This suggests that casein may be linked to the development of the immune system in the early stages of life, and it may be relevant throughout life as a way of activating the immune system; this notion has been demonstrated experimentally by mice that, when injected with lethal doses of bacteria, can survive only after inducing protection by granulocytes with administration of casein [40].

Studies of the effect of casein or sodium caseinate on hematopoietic tissue in vivo and in vitro are limited, and most of them do not include non-casein protein experimental controls. In some cases, there could be controversy in its effects observed due to the presence of a general source of protein or if they were specific to casein. In this sense, it would be more than advisable for future casein work to consider the inclusion of non-casein protein controls.

#### **4. The role of caseins in granulopoiesis and monocytopoiesis**

Caseins and sodium caseinate have been studied for almost four decades, where, from the beginning, it was clear that there was a proinflammatory effect of casein on myeloid lineage cells; this activity was demonstrated by Lotem and Sachs working group, which showed that sodium caseinate had the ability to differentiate a leukemic cell line of myeloid origin toward granulocytes and macrophages

in mice via inflammation and via the activity of T lymphocytes in the peritoneal cavity [41]. Later, this same group showed that the inflammation caused by sodium caseinate had the ability to induce the production of G-CSF and GM-CSF in vitro and in vivo [42]. Another study showed that protein deprivation, such as dietary casein restriction, in rats directly resulted in a decrease in erythropoietin, which is a hormone that is directly related to the proliferation of the erythroid lineage [43]. In a similar study, the role of casein on this lineage was reconfirmed, since the restriction of protein in standard diets in mice once again demonstrated the involvement of proteins in the proliferation of erythroid progenitors in mice [44]. Subsequently, it was shown that after intraperitoneal inoculation of casein, both the production of G-CSF and GM-CSF were rapidly induced, and the high concentration of both cytokines caused a high migration of neutrophils only at the site of inoculation, but they revealed no increase in their percentage in the bone marrow [45]. Interestingly, in a study carried out by the Noursadeghi group, it was shown that after previous inoculation of casein, protection could be given to mice treated with lethal doses of bacteria, and this was due to defensive ability of activated neutrophils recruited by G-CSF induced by that intraperitoneal (IP) injection of casein [40]. Regarding the casein and sodium caseinate fractions on myeloid cell lines, one study showed that in vitro sodium caseinate had the ability to inhibit the proliferation of a myeloid cell line 32D without inhibiting its viability. On the other hand,  $\alpha$ -casein exhibited the ability to inhibit the proliferation of a myeloid tumor line, which is the case for WEHI-3 and sodium caseinate of tumor lines J-774 and P388 at different concentrations. In this same study, it was shown that casein fractions  $\alpha$ -,  $\beta$ - and  $\kappa$ -casein could induce differentiation of the 32D cell line but not the WEHI-3 tumor cell line, but the study interestingly demonstrated that sodium caseinate and  $\alpha$ -casein have the ability to induce M-CSF secretion in the 32D cell line [46]. The same group of researchers subsequently demonstrated that sodium caseinate has the ability to induce the differentiation of the granulocytic lineage in vitro in the same way that G-CSF does, and G-CSF is the specific cytokine required for the differentiation of this lineage; these results demonstrated that these cells have the ability to induce the production of functional M-CSF, a key cytokine in the activation and differentiation of the monocytic lineage [47]. The Vordenbaumen group demonstrated that macrophages of human origin could be stimulated by human  $\alpha$ S1-casein to produce GM-CSF and that  $\alpha$ S1-casein activated the p38 MAPK pathway, which is an important signal in cells of hematopoietic origin. Interestingly, this group found that  $\alpha$ S1-casein was linked to specific receptors for the protein in most of the macrophages analyzed and not only that but also that it could induce the production of IL-1 and IL-6, which positions it as an excellent immunomodulatory protein [48]. This same group subsequently demonstrated that  $\alpha$ S1-casein in human milk has the ability to differentiate human monocytes from macrophages, and they also increase the phagocytic capacity of the monocytes in vitro once stimulated with the protein [49]. On the other hand, the Santiago-Orsorio group showed that the IP inoculation of BALB/c mice with sodium caseinate induces in vivo proliferation of the myeloid lineage in the bone marrow. Interestingly, it was observed that the granulocytes had the ability to incorporate BrdU, a thymidine analog that is incorporated into proliferating cells; further, the cells exhibited a greater ability to phagocytose when compared to cells from mice that had not received treatment [37].

## 5. The role of caseins in lymphopoiesis

Regarding the influence of caseins or sodium caseinate on the lymphoid lineage, the role of these proteins is not prominent, but they have interesting functions

in vivo and in vitro; for instance, the proteolytic activity of leukocytes can be induced by  $\beta$ -casein in vitro [50]. In another work, it was demonstrated that peptides derived from  $\alpha$ S1-casein increased the concentration of IFN- $\gamma$  by stimulating CD8 $^{+}$  T cells, and IFN- $\gamma$  is a potent inhibitor of Th2 lymphocyte-dependent events as well as an inhibitor of the production of IgE [51]. Another working group demonstrated that  $\beta$ -casein rather than  $\kappa$ -casein is mainly responsible for inhibiting spleen CD3 $^{+}$  T lymphocytes [52]. In a more detailed work, the group of Santiago Osorio demonstrated that the IP inoculation of BALB/c mice with sodium caseinate decreases the proliferation of B/B220 $^{+}$  lymphocytes in bone marrow, but this lineage increases proliferation in the spleen; this observation suggests that the IP injection induces extramedullary lymphopoiesis, while the treatment does not increase the proliferation of lineage lymphocytes specifically in the thymus and has only very subtle effects in the spleen without affecting the viability of both lineages. It should be noted that the production of IL-2, IL-7, and IL-15 is increased, and they are key interleukins involved in lymphopoiesis of both T cells and B cells in mice [53]. Although there is currently clear evidence for the role of caseins or sodium caseinate on the lymphoid lineage, there is more evidence of their effect on the myeloid lineage, which may be due to the characteristics of the protein and its particular influence on these cells. However, the field is still open to further exploration of the role of sodium caseinate or caseins on the lymphoid lineage, since its role is not yet clear.

## 6. Inflammation and immune system enhancement by caseins

Milk is a complex physiological liquid that simultaneously provides nutrients and bioactive components, including prebiotics, immune proteins, and the microbiome of human milk itself; the establishment of symbiotic microflora and the development of gut-associated lymphoid tissues facilitate the successful postnatal adaptation of the newborn infant by stimulating cellular growth and digestive maturation [9]. Breastfeeding is associated with a decreased incidence of gastrointestinal (GI) tract infections [54, 55], which is corroborated by several studies that have correlated breastfeeding with a lower incidence of necrotizing enterocolitis in humans and animal models [56, 57].

The antimicrobial activity in milk is greater than the sum individual immunoglobulin and of whey proteins such as lactoferrin, lactoferricins, lactoperoxidase, lysozyme, lactenin, caseinodubins, etc. [58]; this activity could be also associated with gut-colonizing bacteria that prevent adhesion and colonization of pathogenic bacteria while stimulating mucosal cell proliferation and enhancing immune development [59]; a portion of these antimicrobial activities are performed by caseins, most likely  $\kappa$ -casein fucose carbohydrate residues.

Purified human  $\kappa$ -casein inhibits specific adhesion of *Helicobacter pylori* to mucous cells at the human gastric surface. The inhibitory activity is abolished by the oxidation of metaperiodate and is considerably reduced by preincubation with  $\alpha$ -L-fucosidase but not with  $\alpha$ -N-acetylneuraminidase or endo- $\beta$ -galactosidase. Thus, glycosylated  $\kappa$ -casein is likely important for the inhibition of *Helicobacter pylori* adhesion and, therefore, infection. This could explain why breastfeeding may protect against *Helicobacter pylori* infection during early life and how the species-specific glycosylation patterns in human bovine  $\kappa$ -casein partly determine both the narrow host spectrum of this human gastric pathogen and the capacity to resist infection [60].

Unphosphorylated  $\alpha$ S1-casein in breast milk may contribute to the development of the immune system before major colonization of the gut by microbes occurs by



triggering immune responses to potential pathogens, including pathogen-associated molecules such as LPS. Moreover,  $\alpha$ S1-casein by itself gives rise to sustained specific IgG antibody production in individuals who nursed [61]. Early infantile autoantibody production in turn is speculated to confer protection from pathogens [62].

$\alpha$ S1-casein activates the secretion of the proinflammatory cytokines GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-1 $\beta$  (interleukin 1 $\beta$ ), IL-6 (interleukin 6), and chemokine IL-8 (interleukin 8) in human monocytes via the mitogen-activated protein kinase p38 (MAPK-p38) signaling pathway [24, 49].

Human unphosphorylated  $\alpha$ S1-casein induces Toll-like receptor 4 (TLRs) mediated expression of the proinflammatory cytokines IL-1 $\beta$ , GM-CSF, and IL-6 in monocytic cells [63] and induces the differentiation of monocytes toward macrophages [49, 64], but this process is not dependent upon LPS. Interestingly, a posttranslational modification in  $\alpha$ S1-casein (a phosphorylation event) inhibits binding to TLR4, which acts as an off switch for proinflammatory effects [48]. Ectopic expression (outside the mammary gland) of  $\alpha$ S1-casein has been detected in inflamed tissues such as synovial cells and cartilage of rheumatoid arthritis, osteoarthritis, and multiple sclerosis patients [63, 65–67], prostate hyperplasia [68], and lymph nodes of encephalomyelitic mice [67]. Hence,  $\alpha$ S1-casein may constitute an autogenous stimulus that upholds chronic autoimmune inflammation via TLR4 [64].

On the other hand, it is known that casein and sodium caseinate are agents that can induce inflammation when inoculated intraperitoneally, and it has been shown that sodium caseinate and casein can stimulate neutrophils to produce proinflammatory cytokines, such as M-CSF, in vitro [47]. Not only that, but it is known that they can induce in this same lineage signaling pathways involved in inflammation, such as p38 MAPK, which in turn stimulates the production of key cytokines both in inflammation and in hematopoietic cell differentiation processes [69]. However, casein inoculation can induce a rapid accumulation of neutrophils within 3 h due to selective release of mature cells from the bone marrow; then, a significant increase in the concentrations of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) occurs in the peritoneal cavity [45], but the accumulation did not affect the serum values of TNF- $\alpha$ , IL-1 $\beta$ , or IL-6. As demonstrated by Noursadeghi, inflammation induced by casein was associated with higher serum G-CSF concentrations, and administration of an Ab that neutralized this cytokine completely nullified protection against *Escherichia coli* infection after casein pretreatment. Injection of recombinant murine G-CSF between 3 and 24 h before infection conferred the same protection that was provided by casein injection [40].

## 7. Toll-like receptors (TLRs) and caseins

Caseins serve as a source of amino acids but also perform a range of functions, including improving micronutrient bioavailability, stimulating intestinal growth and maturation, supporting immunologic defense, shaping the microbiome, and enhancing learning and memory [19]. Some bioactive peptides in milk act in variable ways as antihypertensives, antithrombotic agents, opioids, antimicrobials, cytomodulators, and immunomodulators [70]. How can this be possible, if proteins are degraded in the gastrointestinal tract to yield the essential amino acids for the development of the neonate?

Bioactive milk peptides were first described in 1950, when Mellander (1950) reported that ingestion of casein-derived phosphorylated peptides led to enhanced vitamin D-independent calcification in rachitic infants. While bioactive peptides



can be generated from a variety of foods, milk proteins are generally regarded as a very rich source; as a result, they have become fundamental constituents of several commercially available functional food products and ingredients [19].

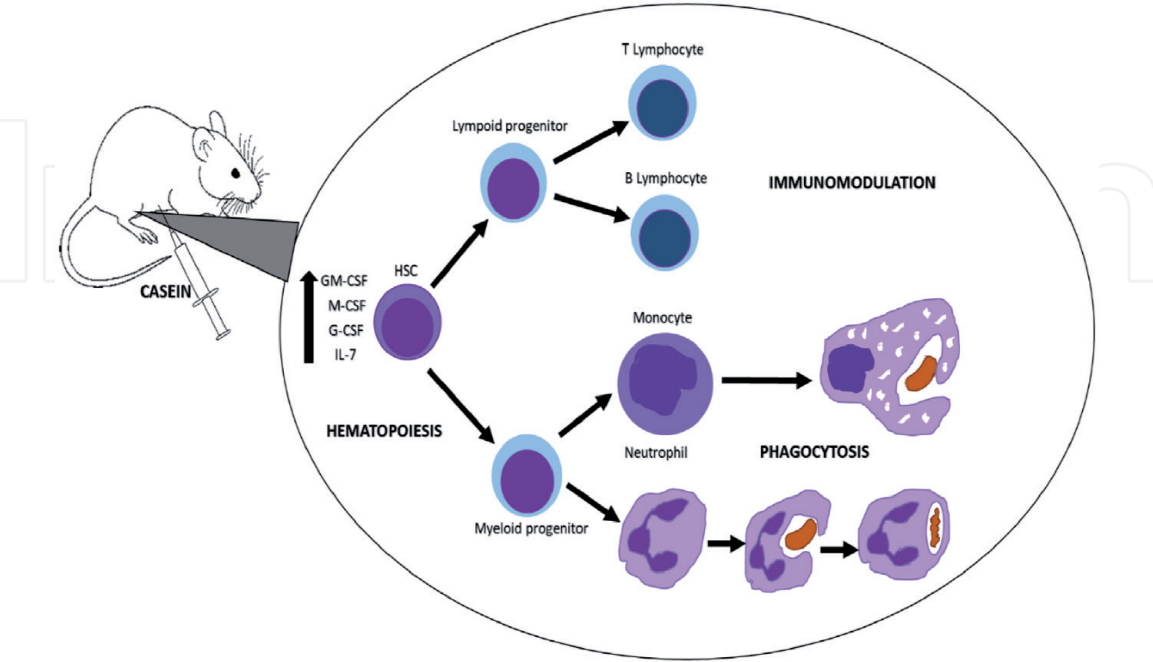
What is the bridge that connects casein, the genesis of myeloid and lymphoid hematopoietic cells, and the activation of the immune system? This linking role may be the direct responsibility of TLRs, which are receptors that recognize at least  $\alpha$ -casein and  $\beta$ -casein [71] and are expressed in granulocytes, macrophages, and B and T lymphocytes; TLRs are capable of activating these cells to produce key cytokines for both proliferation and activation of the innate and adaptive immune response, such as TNF- $\alpha$  [72], G-CSF, IL-2 [73, 74], and IL-7 [75].

In this sense, there is evidence that at least  $\beta$ -casein can influence B lymphocytes via TLR4 [76, 77], and a recent study showed that casein binds directly to TLR4 of CD8+ T lymphocytes [64], although it has also been shown that  $\beta$ -casein can influence the activation and production of histamine through a kinase-dependent mechanism PI3 [78].

Regarding the role of TLR in the production of key cytokines for the activation and proliferation of both T and B lymphocytes, TLR4 of T lymphocytes is involved in the production of IL-2 [73, 74].

For myeloid cells, neutrophils have been shown to use both TLR4 and TLR2 for survival and activation [79]. Thus, TLR4 is essential for the production of G-CSF in neutrophils stimulated with *Clostridium* [80], and another study showed that in addition to G-CSF, TLR4 is capable of inducing the expression of GM-CSF, which plays a fundamental role in the activation and differentiation of both neutrophils and monocytes [81].

TLRs have been shown to be essential for the activation of monocytic cells, and it plays a role in the production of GM-CSF by activating the transcription factor PU.1, which plays a fundamental role in this lineage [82]. In dendritic cells derived from macrophages, TLRs are involved in the synthesis of IL-7, which is an essential interleukin for the maintenance of LT CD8+ [75]. Therefore, casein or sodium



**Figure 1.** Action mechanism of caseins to induce hematopoiesis and immunomodulation. Casein is highly likely to induce TLR4 activation, after which the expression of GM-CSF, G-CSF, M-CSF, and IL-7 is induced in both myeloid and lymphoid cells. This massive cellular activation of hematopoietic cells and immune responses explain the antimicrobial and immunomodulatory effects of casein.

caseinate could stimulate TLR4 via the production of at least IL-7 in dendritic cells derived from macrophages.

It is clear that there is a close relationship between TLR4 and cells of myeloid and lymphoid origin, as these cells use TLR4 both in their proliferation and in their activation. This relationship then entails the production of cytokines of myeloid and lymphoid origin, and these cytokines in turn are key pieces for the proliferation and activation of these same cells. Thus, TLR4-bound caseins or sodium caseinate are highly likely to induce TLR4 activation, after which the expression of GM-CSF, G-CSF, M-CSF, and IL-7 is induced in both myeloid and lymphoid cells (**Figure 1**). It is even possible that indirectly, by stimulating other cell types, TLR4 could induce the activation of cells that can influence the erythroid and megakaryocyte lineage. Then, this massive cellular activation of hematopoietic cells and immune responses could explain the antimicrobial and immunomodulatory effects of casein as well as the activity of casein as an antihypertensive, antithrombotic, and antioxidant molecule [83].

Here, we can reveal that activation of the innate immune system by casein could be useful to fight pathogens resistant to antibiotics, as has been suggested [40], so casein could be used to induce a strong immune response in immunosuppressed patients [84, 85]; it could be used as a prophylactic strategy to prevent infections.

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## References

- [1] Santos EW, Oliveira DC, Silva GB, Tsujita M, Beltran JO, Hastreiter A, et al. Hematological alterations in protein malnutrition. *Nutrition Reviews*. 2017;**75**:909-919. DOI: 10.1093/nutrit/nux041
- [2] Wilkinson AC, Yamazaki S. The hematopoietic stem cell diet. *International Journal of Hematology*. 2018;**107**:634-641. DOI: 10.1007/s12185-018-2451-1
- [3] Mana MD, Kuo EYS, Yilmaz ÖH. Dietary regulation of adult stem cells. *Current Stem Cell Reports*. 2017;**3**:1-8. DOI: 10.1007/s40778-017-0072-x
- [4] Lazare S, Ausema A, Reijne AC, van Dijk G, van Os R, de Haan G. Lifelong dietary intervention does not affect hematopoietic stem cell function. *Experimental Hematology*. 2017;**53**:26-30. DOI: 10.1016/j.exphem.2017.06.002
- [5] Mihaylova MM, Sabatini DM, Yilmaz ÖH. Dietary and metabolic control of stem cell function in physiology and cancer. *Cell Stem Cell*. 2014;**14**:292-305. DOI: 10.1016/j.stem.2014.02.008
- [6] Borelli P, Blatt SL, Rogero MM, Fock RA. Haematological alterations in protein malnutrition. *Revista Brasileira de Hematologia e Hemoterapia*. 2004;**26**:49-56. DOI: 10.1590/s1516-84842004000100010
- [7] Hvatum M, Kanerud L, Hällgren R, Brandtzaeg P. The gut-joint axis: Cross reactive food antibodies in rheumatoid arthritis. *Gut*. 2006;**55**:1240-1247. DOI: 10.1136/gut.2005.076901
- [8] Lönnerdal B, Erdmann P, Thakkar SK, Sauser J, Destallats F. Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: A developmental perspective. *The Journal of Nutritional Biochemistry*. 2017;**41**:1-11. DOI: 10.1016/j.jnutbio.2016.06.001
- [9] Petrotos K, Tsakali E, Goulas P, D'Alessandro AG. Casein and whey proteins in human health. In: *Milk and Dairy Products as Functional Foods*. 2014:94-146. DOI: 10.1002/9781118635056.ch4
- [10] Johnsen LB, Rasmussen LK, Petersen TE, Berglund L. Characterization of three types of human  $\alpha(s1)$ -casein mRNA transcripts. *Biochemical Journal*. 1995;**309**:237-242. DOI: 10.1042/bj3090237
- [11] Ginger MR, Grigor MR. Comparative aspects of milk caseins. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*. 1999;**124**:133-145. DOI: 10.1016/s0305-0491(99)00110-8
- [12] Wang X, Zhao X, Huang D, Pan X, Qi Y, Yang Y, et al. Proteomic analysis and cross species comparison of casein fractions from the milk of dairy animals. *Scientific Reports*. 2017;**7**:1-9. DOI: 10.1038/srep43020
- [13] Uniacke-Lowe T, Fox PF. Equine milk proteins: Chemistry, structure and nutritional significance. *International Dairy Journal*. 2010;**20**:609-629. DOI: 10.1016/j.idairyj.2010.02.007
- [14] Holt C. The milk salts and their interaction with casein. In: *Adv. Dairy Chem*. Vol. 3. Springer US; 1997. pp. 233-256. DOI: 10.1007/978-1-4757-4409-5\_6
- [15] Mølgaard C, Larnkjær A, Arnberg K, Michaelsen KF. Milk and growth in children: Effects of whey and casein. In: *Nestle Nutr. Work. Ser. Pediatr. Progr. Basel: KARGER*; 2011. pp. 67-78. DOI: 10.1159/000325576
- [16] Holt C, Carver JA, Ecroyd H, Thorn DC. Invited review: Caseins and the

casein micelle: Their biological functions, structures, and behavior in foods 1. *Journal of Dairy Science*. 2013;**96**:6127-6146. DOI: 10.3168/jds.2013-6831

[17] Ferretti L, Leone P, Sgaramella V. Long range restriction analysis of the bovine casein genes. *Nucleic Acids Research*. 1990;**18**:6829-6833. DOI: 10.1093/nar/18.23.6829

[18] Day L, Williams RPW, Otter D, Augustin MA. Casein polymorphism heterogeneity influences casein micelle size in milk of individual cows. *Journal of Dairy Science*. 2015;**98**:3633-3644. DOI: 10.3168/jds.2014-9285

[19] Donovan SM. Human Milk Proteins: Composition and Physiological Significance. 2019. pp. 93-101. DOI: 10.1159/000490298

[20] Farrell HM, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, et al. Nomenclature of the proteins of cows' milk—Sixth revision. *Journal of Dairy Science*. 2004;**87**:1641-1674. DOI: 10.3168/jds.S0022-0302(04)73319-6

[21] Swaisgood HE. Chemistry of the caseins. In: *Advanced Dairy Chemistry—1 Proteins*. Boston, MA: Springer; 1992. pp. 63-110

[22] Rasmussen LK, Hojrup P, Petersen TE. Localization of two interchain disulfide bridges in dimers of bovine  $\alpha$ s2-casein. Parallel and antiparallel alignments of the polypeptide chains. *European Journal of Biochemistry*. 1992;**203**:381-386. DOI: 10.1111/j.1432-1033.1992.tb16561.x

[23] Altendorfer I, König S, Braukmann A, Saenger T, Bleck E, Vordenbäumen S, et al. Quantification of  $\alpha$ S1-casein in breast milk using a targeted mass spectrometry-based approach. *Journal of Pharmaceutical and Biomedical Analysis*. 2015;**103**:52-58. DOI: 10.1016/j.jpba.2014.10.034

[24] Vordenbäumen S, Braukmann A, Petermann K, Scharf A, Bleck E, von Mikecz A, et al. Casein  $\alpha$ s1 is expressed by human monocytes and upregulates the production of GM-CSF via p 38 MAPK. *Journal of Immunology*. 2011;**186**:592-601. DOI: 10.4049/jimmunol.1001461

[25] Ng-Kwai-Hang K, Grosclaude F. Genetic polymorphism of milk proteins. In: *Advanced Dairy Chemistry*. Boston, MA: Springer; 1992

[26] Ul Haq MR, Kapila R, Shandilya UK, Kapila S. Impact of milk derived  $\beta$ -casomorphins on physiological functions and trends in research: A review. *International Journal of Food Properties*. 2014;**17**:1726-1741. DOI: 10.1080/10942912.2012.712077

[27] Truswell AS. The A2 milk case: A critical review. *European Journal of Clinical Nutrition*. 2005;**59**:623-631. DOI: 10.1038/sj.ejcn.1602104

[28] Brignon G, Chtourou A, Ribadeau-Dumas B. Preparation and amino acid sequence of human  $\kappa$ -casein. *FEBS Letters*. 1985;**188**:48-54. DOI: 10.1016/0014-5793(85)80872-3

[29] Bergström S, Hansson L, Hernell O, Lönnerdal B, Nilsson AK, Strömquist M. Cloning and sequencing of human  $\kappa$ -casein cDNA. *Mitochondrial DNA*. 1992;**3**:245-246. DOI: 10.3109/10425179209034024

[30] Creamer LK, Plowman JE, Liddell MJ, Smith MH, Hill JP. Micelle stability:  $\kappa$ -casein structure and function. *Journal of Dairy Science*. 1998;**81**:3004-3012. DOI: 10.3168/jds.S0022-0302(98)75864-3

[31] Holt C. Structure and stability of bovine casein micelles. *Advances in Protein Chemistry*. 1992;**43**:63-151. DOI: 10.1016/S0065-3233(08)60554-9

[32] Hastreiter AA, Makiyama EN, Borelli P, Fock RA. Impairment of



G-CSF receptor on granulocytic progenitor cells causes neutropenia in protein malnutrition. *Nutrition*. 2020;**69**:1-8. DOI: 10.1016/j.nut.2019.06.021

[33] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*. 2001;**25**:402-408. DOI: 10.1006/meth.2001.1262

[34] Scrimshaw NS, Viteri FE. INCAP studies of kwashiorkor and marasmus. *Food and Nutrition Bulletin*. 2010;**31**:34-41. DOI: 10.1177/156482651003100105

[35] Keusch GT. The history of nutrition: Malnutrition, infection and immunity. *The Journal of Nutrition*. 2003;**133**:336S-340S. DOI: 10.1093/jn/133.1.336s

[36] Chee LCY, Hendy J, Purton LE, McArthur GA. The granulocyte-colony stimulating factor receptor (G-CSFR) interacts with retinoic acid receptors (RARs) in the regulation of myeloid differentiation. *Journal of Leukocyte Biology*. 2013;**93**:235-243. DOI: 10.1189/jlb.1211609

[37] Domínguez-Melendez V, Silvestre-Santana O, Moreno-Fierros L, Aguiñiga-Sánchez I, Martínez L, Marroquin-Segura R, et al. Sodium caseinate induces mouse granulopoiesis. *Inflammation Research*. 2012;**61**:367-373. DOI: 10.1007/s00011-011-0421-7

[38] Harmon JR, Spengler JR, Coleman-McCray JD, Nichol ST, Spiropoulou CF, McElroy AK. CD4 T cells, CD8 T cells, and monocytes coordinate to prevent rift valley fever virus encephalitis. *Journal of Virology*. 2018;**92**:e01270-18. DOI: 10.1128/jvi.01270-18

[39] Sawa T, Kinoshita M, Inoue K, Ohara J, Moriyama K. Immunoglobulin for treating bacterial infections: One

more mechanism of action. *Antibodies*. 2019;**8**:52. DOI: 10.3390/antib8040052

[40] Noursadeghi M, Bickerstaff MCM, Herbert J, Moyes D, Cohen J, Pepys MB. Production of granulocyte colony-stimulating factor in the nonspecific acute phase response enhances host resistance to bacterial infection. *Journal of Immunology*. 2002;**169**:913-919. DOI: 10.4049/jimmunol.169.2.913

[41] Lotem J, Sachs L. Control of in vivo differentiation of myeloid leukemic cells. III. Regulation by T lymphocytes and inflammation. *International Journal of Cancer*. 1983;**32**:781-791. DOI: 10.1002/ijc.2910320620

[42] Lotem J, Sachs L. Independent regulation of myeloid cell growth and differentiation inducing proteins: In vivo regulation by compounds that induce inflammation. *International Journal of Cancer*. 1985;**35**:93-100. DOI: 10.1002/ijc.2910350115

[43] Okano M, Ohnata H, Sasaki R. Protein deficiency impairs erythropoiesis in rats by reducing serum erythropoietin concentration and the population size of erythroid precursor cells. *The Journal of Nutrition*. 1992;**122**:1376-1383. DOI: 10.1093/jn/122.7.1376

[44] Barceló AC, Alippi RM, Boyer P, Olivera MI, Mide SM, Caro J, et al. Impaired response of polycythemic mice to erythropoietin induced by protein starvation imposed after hormone administration. *Stem Cells*. 1993;**11**:296-302. DOI: 10.1002/stem.5530110407

[45] Metcalf D, Robb L, Dunn AR, Mifsud S, Di Rago L. Role of granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor in the development of an acute neutrophil inflammatory response in mice. *Blood*.

1996;**88**:3755-3764. DOI: 10.1182/blood.v88.10.3755.bloodjournal88103755

[46] Ramos-Mandujano G, Weiss-Steider B, Melo B, Córdova Y, Ledesma-Martínez E, Bustos S, et al. Alpha-, beta- and kappa-caseins inhibit the proliferation of the myeloid cell lines 32D cl3 and WEHI-3 and exhibit different differentiation properties. *Immunobiology*. 2008;**213**:133-141. DOI: 10.1016/j.imbio.2007.07.004

[47] Santiago-Ororio E, Mora L, Bautista M, Montesinos JJ, Martínez I, Ramos-Mandujano G, et al. Sodium caseinate induces secretion of macrophage colony-stimulating factor from neutrophils. *Immunobiology*. 2010;**215**:332-339. DOI: 10.1016/j.imbio.2009.03.003

[48] Vordenbäumen S, Saenger T, Braukmann A, Tahan T, Bleck E, Jose J, et al. Human casein alpha s1 induces proinflammatory cytokine expression in monocytic cells by TLR4 signaling. *Molecular Nutrition & Food Research*. 2016;**60**:1079-1089. DOI: 10.1002/mnfr.201500792

[49] Vordenbäumen S, Braukmann A, Altendorfer I, Bleck E, Jose J, Schneider M. Human casein alpha s1 (CSN1S1) skews in vitro differentiation of monocytes towards macrophages. *BMC Immunology*. 2013;**14**:46. DOI: 10.1186/1471-2172-14-46

[50] Verdi RJ, Barbano DM. Properties of proteases from milk somatic cells and blood leukocytes. *Journal of Dairy Science*. 1991;**74**:2077-2081. DOI: 10.3168/jds.S0022-0302(91)78379-3

[51] Totsuka M, Kakehi M, Kohyama M, Hachimura S, Hisatsune T, Kaminogawa S. Enhancement of antigen-specific IFN- $\gamma$  production from CD8<sup>+</sup> T cells by a single amino acid-substituted peptide derived from bovine  $\alpha$ (s1)-casein. *Clinical Immunology and*

*Immunopathology*. 1998;**88**:277-286. DOI: 10.1006/clin.1998.4585

[52] Bonomi F, Brandt R, Favalli S, Ferranti P, Fierro O, Frøkiær H, et al. Structural determinants of the immunomodulatory properties of the C-terminal region of bovine  $\beta$ -casein. *International Dairy Journal*. 2011;**21**:770-776. DOI: 10.1016/j.idairyj.2011.04.012

[53] Domínguez-Meléndez V, Aguiñiga-Sánchez I, Moreno-Fierros L, Torres B, Osorio ES. El caseinato de sodio incrementa número de linfocitos B en ratones. *Biomédica*. 2017;**37**:571-576. DOI: 10.7705/biomedica.v34i2.3604

[54] Gill HS, Doull F, Rutherford KJ, Cross ML. Immunoregulatory peptides in bovine milk. *British Journal of Nutrition*. 2018;**84**:111-117. DOI: 10.1017/S0007114500002336

[55] M. Knopfler, How Compatible is Cow's Milk with the Human Immune System?, 2016. Available from: <https://touro scholar.touro.edu/sjlcas> [Accessed: 31 January 2020]

[56] Meinen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. *Journal of Perinatology*. 2009;**29**:57-62. DOI: 10.1038/jp.2008.117

[57] Sodhi C, Richardson W, Gripar S, Hackam DJ. The development of animal models for the study of necrotizing enterocolitis. *Disease Models & Mechanisms*. 2008;**1**:94-98. DOI: 10.1242/dmm.000315

[58] Park YW. Bioactive peptides in milk and dairy products: A review. *Korean Journal for Food Science of Animal Resources*. 2015;**35**:831-840. DOI: 10.5851/kosfa.2015.35.6.831

[59] Ward TL, Hosid S, Ioshikhes I, Altosaar I. Human milk metagenome: A functional capacity analysis.

BMC Microbiology. 2013;**13**:1. DOI: 10.1186/1471-2180-13-116

[60] Hernell O, Hansson L. Human milk  $\kappa$ -casein and inhibition of helicobacter pylori adhesion to human gastric mucosa. *Journal of Pediatric Gastroenterology and Nutrition*. 1995;**21**:288-296. DOI: 10.1097/00005176-199510000-00006

[61] Petermann K, Vordenbäumen S, Maas R, Braukmann A, Bleck E, Saenger T, et al. Autoantibodies to  $\alpha$ s1-casein are induced by breast-feeding. *PLoS One*. 2012;**7**:e32716. DOI: 10.1371/journal.pone.0032716

[62] Barbouche R, Forveille M, Fischer A, Avrameas S, Durandy A. Spontaneous IgM autoantibody production in vitro by B lymphocytes of normal human neonates. *Scandinavian Journal of Immunology*. 1992;**35**:659-667. DOI: 10.1111/j.1365-3083.1992.tb02972.x

[63] Galligan CL, Baig E, Bykerk V, Keystone EC, Fish EN. Distinctive gene expression signatures in rheumatoid arthritis synovial tissue fibroblast cells: Correlates with disease activity. *Genes and Immunity*. 2007;**8**:480-491. DOI: 10.1038/sj.gene.6364400

[64] Saenger T, Vordenbäumen S, Genich S, Haidar S, Schulte M, Nienberg C, et al. Human  $\alpha$ S1-casein induces IL-8 secretion by binding to the ecto-domain of the TLR4/MD2 receptor complex. *Biochimica et Biophysica Acta, General Subjects*. 2019;**1863**:632-643. DOI: 10.1016/j.bbagen.2018.12.007

[65] Ungethuem U, Haeupl T, Witt H, Koczan D, Krenn V, Huber H, et al. Molecular signatures and new candidates to target the pathogenesis of rheumatoid arthritis. *Physiological Genomics*. 2010;**42A**:267-282. DOI: 10.1152/physiolgenomics.00004.2010

[66] Karlsson C, Dehne T, Lindahl A, Brittberg M, Pruss A, Sittlinger M, et al. Genome-wide expression profiling

reveals new candidate genes associated with osteoarthritis. *Osteoarthritis and Cartilage*. 2010;**18**:581-592. DOI: 10.1016/j.joca.2009.12.002

[67] Otaegui D, Mostafavi S, Bernard CCA, de Munain AL, Mousavi P, Oksenberg JR, et al. Increased transcriptional activity of milk-related genes following the active phase of experimental autoimmune encephalomyelitis and multiple sclerosis. *Journal of Immunology*. 2007;**179**:4074-4082. DOI: 10.4049/jimmunol.179.6.4074

[68] Xu K, Ling MT, Wang X, Wong YC. Evidence of a novel biomarker,  $\alpha$ s1-Casein, a milk protein, in benign prostate hyperplasia. *Prostate Cancer and Prostatic Diseases*. 2006;**9**:293-297. DOI: 10.1038/sj.pcan.4500872

[69] Van Overmeire E, Stijlemans B, Heymann F, Keirsse J, Morias Y, Elkrim Y, et al. M-CSF and GM-CSF receptor signaling differentially regulate monocyte maturation and macrophage polarization in the tumor microenvironment. *Cancer Research*. 2016;**76**:35-42. DOI: 10.1158/0008-5472.CAN-15-0869

[70] Hayes M, Stanton C, Fitzgerald GF, Ross RP. Putting microbes to work: Dairy fermentation, cell factories and bioactive peptides. Part II: Bioactive peptide functions. *Biotechnology Journal*. 2007;**2**:435-449. DOI: 10.1002/biot.200700045

[71] Lewis SL, Van Epps DE. Demonstration of specific receptors for fluoresceinated casein on human neutrophils and monocytes using flow cytometry. *Inflammation*. 1983;**7**:363-375. DOI: 10.1007/BF00916301

[72] Arruda-Silva F, Bianchetto-Aguilera F, Gasperini S, Polletti S, Cosentino E, Tamassia N, et al. Human neutrophils produce CCL23 in response to various TLR-agonists and TNF $\alpha$ . *Frontiers in Cellular and Infection*



Microbiology. 2017;7:176. DOI: 10.3389/fcimb.2017.00176

[73] Rossato L, dos Santos SS, Ferreira LG, de Almeida SR. The importance of Toll-like receptor 4 during experimental *Sporothrix brasiliensis* infection. Medical Mycology. 2019;57:489-495. DOI: 10.1093/mmy/myy048

[74] Seydoux E, Liang H, Dubois Cauwelaert N, Archer M, Rintala ND, Kramer R, et al. Effective combination adjuvants engage both TLR and inflammasome pathways to promote potent adaptive immune responses. Journal of Immunology. 2018;201:98-112. DOI: 10.4049/jimmunol.1701604

[75] Carreno BM, Becker-Hapak M, Linette GP. CD40 regulates human dendritic cell-derived IL-7 production that, in turn, contributes to CD8<sup>+</sup> T-cell antigen-specific expansion. Immunology and Cell Biology. 2009;87:167-177. DOI: 10.1038/icb.2008.80

[76] Tobita K, Kawahara T, Otani H. Bovine  $\beta$ -casein (1-28), a casein phosphopeptide, enhances proliferation and IL-6 expression of mouse CD19<sup>+</sup> cells via toll-like receptor 4. Journal of Agricultural and Food Chemistry. 2006;54:8013-8017. DOI: 10.1021/jf0610864

[77] Kawahara T, Katayama D, Otani H. Effect of-casein (1-28) on proliferative responses and secretory functions of human immunocompetent cell lines. Bioscience, Biotechnology, and Biochemistry. 2004;68:2091-2095

[78] Smuda C, Wechsler JB, Bryce PJ. TLR-induced activation of neutrophils promotes histamine production via a PI3 kinase dependent mechanism. Immunology Letters. 2011;141:102-108. DOI: 10.1016/j.imlet.2011.08.002

[79] Sabroe I, Prince LR, Jones EC, Horsburgh MJ, Foster SJ, Vogel SN, et al. Selective roles for Toll-like receptor (TLR)2 and TLR4 in the regulation

of neutrophil activation and life span. Journal of Immunology. 2003;170:5268-5275. DOI: 10.4049/jimmunol.170.10.5268

[80] Takehara M, Seike S, Sonobe Y, Bandou H, Yokoyama S, Takagishi T, et al. Clostridium perfringens  $\alpha$ -toxin impairs granulocyte colony-stimulating factor receptor-mediated granulocyte production while triggering septic shock. Communications Biology. 2019;2:1-12. DOI: 10.1038/s42003-019-0280-2

[81] Hayashi F, Means TK, Luster AD. Toll-like receptors stimulate human neutrophil function. Blood. 2003;102:2660-2669. DOI: 10.1182/blood-2003-04-1078

[82] Sadeghi K, Wisgrill L, Wessely I, Diesner SC, Schuller S, Dürr C, et al. GM-CSF down-regulates TLR expression via the transcription factor PU.1 in human monocytes. PLoS One. 2016;11:e0162667. DOI: 10.1371/journal.pone.0162667

[83] Meisel H, Bockelmann W. Bioactive peptides encrypted in milk proteins: Proteolytic activation and thropho-functional properties. In: van Leeuwenhoek A editor. Int. J. Gen. Mol. Microbiol, 1999. pp. 207-215. DOI: 10.1023/A:1002063805780

[84] Millon L, Manteaux A, Reboux G, Drobacheff C, Monod M, Barale T, et al. Fluconazole-resistant recurrent oral candidiasis in human immunodeficiency virus-positive patients: Persistence of Candida albicans strains with the same genotype. Journal of Clinical Microbiology. 1994;32:1115-1118. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/8027327> [Accessed: 31 January 2020]

[85] Popa LG, Popa MI, Mihai IR. Case-control study to evaluate the link between immunosuppression and Candida spp. infection. Roumanian Archives of Microbiology and Immunology. 2005;64:72-76