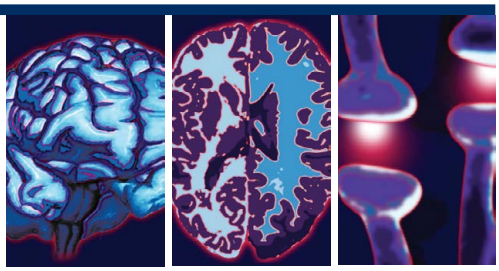
Review



Development of colostrum MAF and its clinical application

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ABSTRACT

Recently, immunotherapy has emerged as a new and appealing strategy for cancer treatment and various other acute and chronic diseases. Essential components of the natural immune system-phagocytic cells called macrophages-multiply in response to an infection in the body. The use of a macrophage activating therapy, such as macrophage activating factor (MAF), has extensive applications for treating numerous diseases by activating the natural macrophages of the body to stimulate the immune system. The aim of this review is to provide insight into the features and clinical efficacy of a new type of macrophage-activating factor derived from colostrum, called colostrum MAF.

Keywords

Immunotherapy, Macrophage Activating Factor (MAF), Colostrum MAF

Introduction

Many diseases, including cancer and AIDS, develop because of the compromise or failure of the natural immune system. Currently, researchers have learned the stimulation of the natural immune system can arrest or even reverse diseases, such as cancer and AIDS. Recently, the new strategy of immunotherapy has become an appealing strategy for the treatment of cancer, as well as the treatment of various other acute and chronic diseases [1].

The body's basic self-defense against disease is the immune system [2]. The immune system can perceive numerous pathogens in the environment, including tumor cells within the body. The immune system's method of protection consists of two categories of immunity: innate immunity and adaptive immunity. Several types of cells exist in the innate immune system, including phagocytic neutrophils, macrophages, dendritic cells, mast cells, and natural killer cells. The adaptive immune system consists of lymphocytes, including both T cells and B cells, which can distinguish and memorize specific pathogens and their products, including antibodies.

An essential component of the innate immune system, macrophages are phagocytic cells that multiply in response to an infection within the body. Macrophages distinguish, overwhelm, and obliterate pathogens, cancer cells, and foreign substances. These macrophages also circulate cytokines and eliminate cellular debris and cells that have undergone apoptosis [3]. Broadly, macrophages are divided into two classes: tissue-resident macrophages and infiltrating macrophages. Most of the body's tissues have tissue-resident macrophage populations [4].

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Intestinal macrophages of the gastrointestinal tract, Langerhans cells, dermal macrophages, Kupffer cells, motile liver macrophages, brain microglia, alveolar and interstitial lung macrophages, spleen red pulp macrophages, and bone marrow macrophages are examples of tissue-resident macrophages. By definition, all these macrophages exist in their respective organ tissues and execute homeostatic tissue-specific functions [4-6]. The source of infiltrating macrophages-inflammatory monocytes-move selectively to areas of inflammation, manufacture inflammatory cytokines, and contribute to both local and systemic inflammation [7]. Infiltrating macrophages occur in pathological environments, including: cancer, atherosclerosis, and metabolic diseases [6]. Macrophages have an important function in wound healing and skin repair. They add to the stimulation of epithelial stem cells and the cyclic stimulation of hair follicle stem cells. The findings could have adaptive implications for skin repairs, hair regrowth, and inflammatory skin diseases [8].

Known as a vitamin D binding protein-macrophage activating factor (DBP-MAF), Gc protein-derived macrophage activating factor (GcMAF) is a potent endogenous macrophage activator that exists naturally in blood. Recently, MAF has been found to offer health benefits. The purpose of this review is to create awareness about the concepts and clinical efficacy of a new type of macrophage-activating factor currently being developed from colostrum (colostrum MAF).

The preceding MAF

■ First generation GcMAF (Purified GcMAF)

Dr. Nobuto Yamamoto discovered GcMAF in 1991. GcMAF is a derivative of the group-specific component (Gc) protein (vitamin D binding protein), which is a component of the albumin superfamily [9]. Purified GcMAF, the first-generation GcMAF, was made using artificial enzymatic treatments of non-specific human Gc protein, which was purified by vitamin D-affinity chromatography. GcMAF is a remarkable serum glycoprotein with numerous biological activities. During an inflammatory response in the body, GcMAF is produced when sialidase of a T-cell and cegalactosidase of an activated B-cell hydrolyze the terminal galactose and sialic acid saccharides of Gc protein [10]. GcMAF exhibits a remarkable biological activity; GcMAF activates macrophages using superoxide radical generation and phagocytic activation [10,11], and *in vivo*, has shown anti-angiogenic [12,13] and anti-tumor [14-16] properties. Additionally, GcMAF directly inhibits propagation and migration of human prostate cancer cells or human breast cancer cells, independently from its macrophage activation abilities [17].

In patients with metastatic breast cancer [18], prostate cancer [19], and metastatic colorectal cancer [20], clinical trials have been conducted using GcMAF. Significantly, among all patients who were administered weekly doses of 100 ng of GcMAF during a 7 to 19-week time frame, cancer did not reappear within a four to seven year period. However, some problems existed with the clinical trials because no apparent classifications of patient histopathological types, grades, and stages were made. Also, the curative conclusions were based exclusively on patient N-acetylgalactosaminidase (Nagalase) activities. In the clinical trials, no measurements were taken of the tumor markers or cytokine levels, and no control group existed.

Additionally, a noteworthy clinical report of HIV treatment using GcMAF [21] was made. Based on a weekly administration of 100 ng of GcMAF to 15 non-anemic HIV-infected patients, results showed the number of CD4+ cells increased to normal levels, the number of CD8+ cells decreased to normal levels, and often within 6 weeks, the amount of HIV-1 RNA and p24 antigen were imperceptible in the patient's blood tests. This report considered that the positive effect of the destruction of the HIV was potentially caused by the GcMAF-activated macrophages phagocytosing and destroying the virus. GcMAF was active in the monocytes and macrophages isolated from these patients with AIDS [22].

■ Second generation GcMAF (Serum GcMAF)

A significant problem has been associated with purification of first-generation GcMAF for clinical use. In previous research, an affinity column modified with 25-hydroxy-vitamin D3 was used to produce purified GcMAF [23]. However, contamination is difficult to avoid when a column is repeatedly used. When at room temperature-in an environment with oxygen, and in the absence of antioxidants, such as albumin and uric acid, which are plentiful in blood-purified GcMAF is unstable [24]. To overcome the stability issue with first-generation GcMAF, second-generation GcMAF is

produced using artificial enzymatic treatment of human serum without the purification that uses vitamin D-affinity chromatography. In mice, the artificial enzymatic treatment enhanced the phagocytic activity of peritoneal macrophages and extended the survival period among mice with Ehrlich ascites tumors [25]. In the case of reports, second generation GcMAF-based immunotherapy partnered with numerous other therapies that were useful in treating cancer patients [26,27].

Autism spectrum disorders (ASD) are neurodevelopmental diseases distinguished by symptoms of restricted interests, repetitive behaviors, and lack of language and social skills [28]. While no specific biological markers exist for autism and ASD, people with ASD and their family members often describe immune system anomalies [29]. The current hypothesis is some combination of immune factors, including maternally developed antibodies to fetal brain tissue, tends to set up microglia so as to prevent the normal functions of directing neuronal migration and pruning [30,31]. In a clinical study of autistic individuals, second generation GcMAF was able to normalize the experiential differences of dysregulated gene expression of the endocannabinoid system in patients' cultured blood monocyte-derived macrophages (BMDMs) [32]. Based on assessments of intelligence, cognition, and behavior of the autistic patients in the study, further studies involving these evaluations are needed.

Colostrum MAF

■ Bovine colostrum

During the first several days post-parturition, cows produced a type of milk called bovine colostrum for their newborn calves. The bovine colostrums, a thick, sticky, yellow-colored liquid, contain serum proteins and antibodies, including albumin, insulin-like growth factor, epidermal growth factor, nerve growth factor, lactoferrin, immunoglobulin G, immunoglobulin A, and immunoglobulin M, which all serve to protect newborn calves from various infectious diseases; however, unlike mature milk, bovine colostrums contain lower amounts of carbohydrates and lipids [33]. Scientists have known the beneficial attributes of colostrum for centuries, but only recently have researchers analyzed the biological components that account for the unique actions of colostrum [34,35]. Notably, all newborns have subtle and immature gastrointestinal systems, and colostrum is used to provide naturally created nutrients in a potent but low-volume process. Additionally, colostrum properties also act to protect and mature the gastrointestinal tract [36]. Colostrum is also used to transfer maternal immunity to the newborn, based on immunoglobulin [37] and also based on factors of their inborn systems [38].

■ Preparation of colostrum MAF

Immunoglobulin A (IgA) is known to provide protection from a variety of infections and interacts with the Fc receptor (Fction from a variety of inflammatory effects [39]. Additionally, IgA has an O-linked sugar chain, and the binding property for the Fc receptor is reduced if many sialic acid residues exist [40]. Reportedly, the number of N-acetylgalactosamine moieties attached to IgA O-linked glycans was significantly reduced in Crohn's patients and correlated strongly with clinical activity [41].

Additionally, Gc protein has an O-linked sugar chain. The hydrolysis of galactose and sialic acid of the Gc protein causes inflammation and is mediated by membrane-bound d galactosidase that exists on activated B-cells and sialidase on T-cells to produce GcMAF with a GalNAc moiety [10]. Using the hypothesis that bovine colostrum could be utilized as a macrophage activator if enzymatically modified IgA and Gc protein exhibited activity similar to the activity GcMAF, one study prepared galactosylated and desialylated bovine colostrum and showed it had the ability to activate macrophage phagocytosis [43]. Thus, colostrum MAF, a new form of a macrophage-activating factor, has been developed.

■ Features of colostrum MAF

Previous studies show that colostrum MAF greatly enhanced phagocytic activity in the peritoneal macrophages and intestinal macrophages of mice, *in vitro* and *in vivo* respectively [42]. The consensus has been that materials with a molecular weight over 500 Da do not undergo intestinal absorption. In contrast, it has been reported that, in mice intestinal tracts, peptides of essentially high molecular weight (~15,000 Da) could be absorbed [43]. Therefore, these findings suggest that glycoprotein in the bovine colostrum with a high molecular weight can be absorbed from the intestinal tract.

To activate macrophages in the gut-associated lymphoid tissue (GALT), colostrum MAF is dispensed in the mouth along with Waldeyer's tonsillar ring **(Figure 1)**. Additionally, colostrum

MAF may be administered in other areas of the body where macrophages exist. In particular, GALT is considered the largest macrophage pool in the body, having an essential role in maintaining and regulating mucosal immunity [44,45]. Thus, it has been shown that oral colostrum MAF-within an acid-resistant enteric-coated capsule-has a potential effect for directly activating a large number of macrophages in GALT to stimulate the immune system.

Colostrum MAF has particular advantages over MAF that is produced from serum, regarding practical clinical use-because colostrum MAF is derived from bovine colostrum, a food source, instead of a human serum source-and it is administered orally and sublingually, instead of by invasive injection.

Additionally, colostrum MAF did not mediate production of inflammatory cytokines, including: tumor necrosis factor-tory cytokine interleukin-1s factoβ) [42]. If colostrum MAF can be used to suppress the production of inflammatory cytokines, it can be an effective treatment for autoimmune diseases.

Effects of colostrum MAF

The effect of colostrum MAF on chronic fatigue syndrome

Chronic fatigue syndrome (CFS) is a complex illness distinguished by unexplained fatigue lasting for at least six months, with symptoms including: headaches, poor sleep, muscle pain, and the cognitive difficulties of memory and concentration problems [46]. Additionally, there are a host of other symptoms that CFS patients may experience. These can affect the senses, cause irritable bowel syndrome, and give psychological disturbances [47]. The exact pathogenesis of CFS is unknown. Several etiological models have been presented, including a role for infection, oxidative and nitrosative (IO&NS) pathways [48], endocrine dysfunction, autonomic nervous system imbalance, depressed mood, and decreased immunity [49]. Low ATP production and mitochondrial dysfunction have an important part in autoimmunity by inhibiting apoptosis and stimulating necrotic cell death [50]. These can cause neurological aberrations including behavioral responses

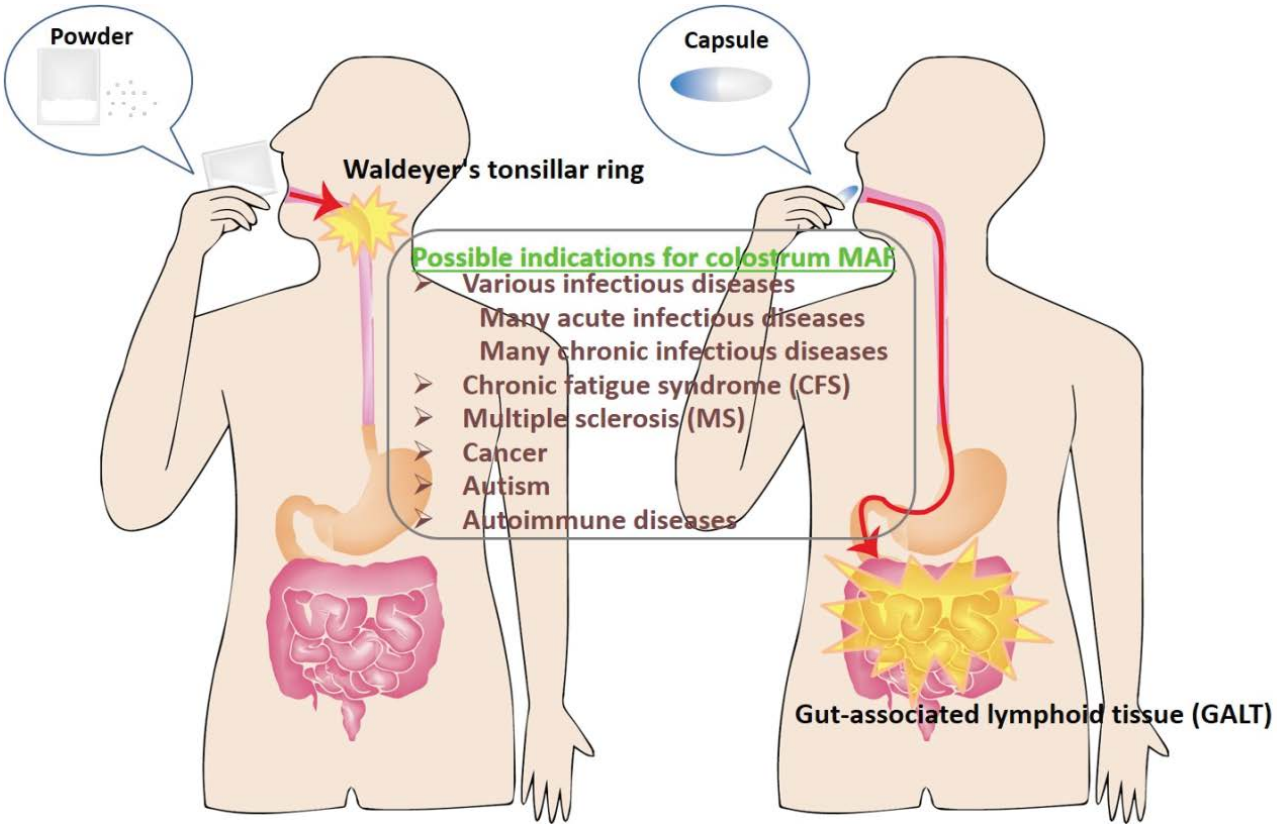

Figure 1: Colostrum MAF is able to reach a target tissue such as Waldeyer's tonsillar ring or GALT by changing dosage form. MAF: macrophage activating factor, GALT: gut-associated lymphoid tissue.

and neuroendocrine, autonomic, and brain dysfunctions. Numerous patients with CFS may experience autoimmune responses, loss of natural killer cell functions, and raised levels of the anti-inflammatory cytokines [51]. Multiple cytokine abnormalities were reported in CFS patients, including: IL-1, TNFα, and IL-6 [52], INFγ 54[53] nuclear factor κB (NF-κB) [54], and the protein kinase R (PKR) pathways [55].

No prescription drugs have been developed specifically for CFS; therefore, it is considered an incurable disease. In our previous case reports, we showed two patients with CFS who had excellent responses with the use of oral colostrum MAF [56]. Specifically, the daily oral administration of colostrum MAF powder and dispensing one acid-resistant, enteric-coated capsule to two CFS patients showed various symptoms-including fatigue, muscle pain, and stomach pain-improved within a few days. Additionally, CFS patients experienced hair regrowth on their heads within a 4-month period.

While the cause of CFS has not yet been identified, previous research indicated that infections and immune dysfunction might have a critical role in the development of CFS. Recently, we reported that macrophage activation with GcMAF-based immunotherapy, unlike lipopolysaccharide (LPS), does not cause nitric oxide (NO) and tumor necrosis factor (TNF)-α, and IL-1β cytokine production [15,42]. According to Uto, *et al.* 10 ng doses of colostrum MAF led to significantly higher macrophage phagocytic activity than typical LPS treatments of 1 μg. As such, microphages appear to show a greater affinity for activation with colostrum MAF than LPS.

The administration of colostrum MAF will result in LPS-related macrophage activation suppression. Therefore, it will induce a good phagocytosis without dysfunction of either IL-1β or TNF-α. The results of these initial experiments with colostrum MAF therapy appear to be connected with the reduction of fatigue in two CFS patients.

In certain cases, colostrum MAF was administered using two methodologies: powder taken orally and one acid-resistant, enteric-coated capsule containing colostrum MAF. The capsule method facilitates the colostrum MAF reaching the gut where it can activate macrophages in the Payer's patches and then enter the blood. The possibility exists that colostrum MAF can be administered through sublingual absorption for introduction into the bloodstream. The case reports indicated that the colostrum MAF molecule could be absorbed using either route, providing effects which appear similar to injected GcMAF, and allowing for a new protocol for the treatment of CFS that uses food-based colostrum MAF.

■ The effect of colostrum MAF on other diseases

Multiple sclerosis (MS) is an idiopathic inflammatory disease distinguished by demyelination and degeneration of the central nervous system (CNS) [57]. The primary role of T-cells in the pathogenesis of MS has long been known [58]. Additionally, B cells have an important part in the pathogenesis of MS [59]. The findings indicated that abnormal interactions between T cells and B cells are implicated in the immunopathogenesis of MS [60]. Based on the limited effective treatment options available for MS, one study treated a 45-year-old male MS patient with serum GcMAF and colostrum MAF [61]. After treatment, the patient exhibited increased energy and, after four years of being confined to a wheelchair, was able to walk. Importantly, all medications for pain and urinary bladder control and antibiotics were discontinued, and the patient could navigate stairs [61]. An MS case study showed that treatment with the second generation GcMAF and colostrum MAF markedly improved the motor ability. The study suggests that using immunomodulation via MAF could be beneficial in the treatment of MS. In another case, oral colostrum MAF and serum GcMAF immunotherapy were combined with son dynamic therapy (SDT), tumor treating fields (TTF), and ozone therapy, and these proved to be effective in when treating a patient who had non-small-cell lung cancer (stage 3B) [62].

Conclusion

Several researchers have explained the effects of MAF on a myriad of diseases **(Table 1)**. Colostrum MAF has multiple positive attributes, including being a safe food, easy to obtain, and a non-inducer of inflammatory cytokines, and it has also been shown to reach target tissues-such as Waldeyer's tonsillar ring or GALT-by changing administration forms. Therefore, colostrum MAF is promising as an effective macrophage activator for various immunotherapies. As such, an additional collection of biological and psychological data from clinical applications is needed to confirm the effects of colostrum MAF in the pathology of different diseases.

Table 1: Efficacy of macrophage-activating factor (MAF).

Formula	Model	Reported outcome	Study	References
Purified GcMAF	Gc protein from human serum	Enhancement of macrophage activity	*In vitro*	Mohamad, *et al.* 2002 [63]
Purified GcMAF	Gc protein from human serum	Enhancement of macrophage phagocytic activity	*In vitro*	Nagasawa, *et al.* 2004 [64]
Purified GcMAF	Several endothelial cells	Antiangiogenic effects by mediating through the CD36 receptor	*In vitro*	Kanda, *et al.* 2002 [12]
Purified GcMAF	Pancreatic cancer	Antiangiogenic effects and tumor regression	*In vitro* and *vivo*	Kisker, *et al.* 2003 [13]
Purified GcMAF	Ehrlich tumor carcinoma	Eradication of ascites tumor	*In vivo*	Koga, *et al.* 1999 [14]
Purified GcMAF	L-929 cell	Anti-tumor actibity	*In vitro*	Mohamad, *et al.* 2003 [15]
Purified GcMAF	Hepatocellular carcinoma	Anti-angiogenic activity and tumor killing activity	*In vivo*	Nonaka, *et al.* 2012 [16]
Purified GcMAF	Breast cancer	Inhibition of tumour-induced angiogenesis and inhibition of cancer cell proliferation, migration and metastatic potential	*In vitro*	Pacini, *et al.* 2012 [17]
Purified GcMAF	Metastatic breast cancer	No recurrence for more than 4 years	Clinical	Yamamoto, *et al.* 2008 [18]
Purified GcMAF	Prostate Cancer	No recurrence occurred for 7 years	Clinical	Yamamoto, *et al.* 2008 [19]
Purified GcMAF	Metastatic colorectal cancer	No recurrence occurred for 7 years	Clinical	Yamamoto, *et al.* 2008 [20]
Purified GcMAF	HIV infection	Increase in number of $CD4^+$cells and decrease in quantity of p24 Antigen and HIV-1 RNA	Clinical	Yamamoto, *et al.* 2009 [21]
Serum GcMAF	Ehrlich tumor carcinoma	Enhancement of macrophage phagocytic activity and extension of the survival time of mice bearing Ehrlich ascites tumors	*In vitro* and *vivo*	Kuchiike, *et al.* 2013 [25]
Serum GcMAF	Metastatic cancers	No progression of cancer	Clinical	Inui, *et al.* 2013 [26]
Serum GcMAF	Metastatic breast cancer	Complete disappearance of the pleural effusion and intra-pleural nodular tumor	Clinical	Inui, *et al.* 2014 [27]
Serum GcMAF	Autism	Normalization in dysregulated gene expression of the endocannabinoid system in cultured blood monocyte-derived macrophages (BMDMs)	Clinical	Siniscalco, *et al.* 2014 [32]
Colostrum MAF	Mouse macrophages	Enhancement of the phagocytic activity of mouse peritoneal macrophages in vitro and of intestinal macrophages + in vivo	*In vitro* and vivo	Uto, *et al.* 2015 [42]
Colostrum MAF	Choronic fatigue syndrome	Improvement of various symptoms	Clinical	Inui, *et al.* 2015 [56]
Colostrum MAF	Multiple sclerosis	Improvement of motor disability and getting off all medication	Clinical	Inui, *et al.* 2016 [61]
Colostrum MAF	Non-small cell lung cancer (stage 3B)	Improvement of various symptoms and inhibition of increase in tumor size	Clinical	Inui, *et al.* 2016 [62]

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Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

References

1. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature* 480(7378), 480-489 (2011).
2. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 140(6), 883-899 (2010).
3. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol* 8(12), 958-969 (2008).
4. Davies LC, Jenkins SJ, Allen JE, *et al.* Tissue-resident macrophages. *Nat. Immunol* 14(10), 986-995 (2013).
5. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol* 11(11), 723-737 (2011).
6. Hashimoto D, Chow A, Noizat C, *et al.* Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* 38(4), 792-804 (2013).
7. Yang J, Zhang L, Yu C, *et al.* Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. *Biomark. Res* 2(1), 1

(2014).

8. Castellana D, Paus R, Perez-Moreno M. Macrophages contribute to the cyclic activation of adult hair follicle stem cells. *PLoS. Biol* 12(12), e1002002 (2014).
9. Yamamoto N, Homma S. Vitamin D3 binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. *Proc. Natl. Acad. Sci. U S A* 88(19), 8539-8543 (1991).
10. Yamamoto N, Kumashiro R. Conversion of vitamin D3 binding protein (group-specific component) to a macrophage activating factor by the stepwise action of beta-galactosidase of B cells and sialidase of T cells. *J. Immunol* 151(5), 2794-2802 (1993).
11. Naraparaju VR, Yamamoto N. Roles of beta-galactosidase of B lymphocytes and sialidase of T lymphocytes in inflammation-primed activation of macrophages. *Immunol. Lett* 43(3), 143-148 (1994).
12. Kanda S, Mochizuki Y, Miyata Y, *et al.* Effects of vitamin D(3)-binding protein-derived macrophage activating factor (GcMAF) on angiogenesis. *J. Natl. Cancer. Inst* 94(17), 1311-1319 (2002).
13. Kisker O, Onizuka S, Becker CM, *et al.* Vitamin D binding protein-macrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. *Neoplasia* 5(1), 32-40 (2003).
14. Koga Y, Naraparaju VR, Yamamoto N. Antitumor effect of vitamin D-binding protein-derived macrophage activating factor on Ehrlich ascites tumor-bearing mice. *Proc. Soc. Exp. Biol. Med* 220(1), 20-26 (1999).
15. Mohamad SB, Nagasawa H, Sasaki H, *et al.* Gc protein-derived macrophage activating factor (GcMAF): isoelectric focusing pattern and tumoricidal activity. *Anticancer. Res* 23(6a), 4451-4457 (2003).
16. Nonaka K, Onizuka S, Ishibashi H, *et al.* Vitamin D binding protein-macrophage activating factor inhibits HCC in SCID mice. *J. Surg. Res* 172(1), 116-122 (2012).
17. Pacini S, Punzi T, Morucci G, *et al.* Effects of vitamin D-binding protein-derived macrophage-activating factor on human breast cancer cells. *Anticancer. Res* 32(1), 45-52 (2012).
18. Yamamoto N, Suyama H, Yamamoto N, *et al.* Immunotherapy of metastatic breast cancer patients with vitamin D-binding protein-derived macrophage activating factor (GcMAF). *Int. J. Cancer* 122(2), 461-467 (2008).
19. Yamamoto N, Suyama H, Yamamoto N. Immunotherapy for Prostate Cancer with Gc Protein-Derived Macrophage-Activating Factor, GcMAF. *Transl. Oncol* 1(2), 65-72 (2008).
20. Yamamoto N, Suyama H, Nakazato H, *et al.* Immunotherapy of metastatic colorectal cancer with vitamin D-binding protein-derived macrophage-activating factor, GcMAF. *Cancer. Immunol. Immunother* 57(7), 1007-1016 (2008).
21. Yamamoto N, Ushijima N, Koga Y. Immunotherapy of HIV-infected patients with Gc protein-derived macrophage activating factor (GcMAF). *J. Med. Virol* 81(1), 16-26 (2009).
22. Yamamoto N, Naraparaju VR, Srinivasula SM. Structural modification of serum vitamin D3-binding protein and immunosuppression in AIDS patients. *AIDS. Res. Hum. Retroviruses* 11(11), 1373-1378 (1995).
23. Swamy N, Roy A, Chang R, *et al.* Affinity purification of human plasma vitamin D-binding protein. Protein. *Expr. Purif* 6(2), 185-188 (1995).
24. Ames BN, Cathcart R, Schwiers E, *et al.* Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc. Natl. Acad. Sci. U S A* 78(11), 6858-6862 (1981).
25. Kuchiike D, Uto Y, Mukai H, *et al.* Degalactosylated/desialylated human serum containing GcMAF induces macrophage phagocytic activity and in vivo antitumor activity. *Anticancer. Res* 33(7), 2881-2885 (2013).
26. Inui T, Kuchiike D, Kubo K, *et al.* Clinical experience of integrative cancer immunotherapy with GcMAF. *Anticancer. Res* 33(7), 2917-2919 (2013).
27. Inui T, Makita K, Miura H, *et al.* Case report: A breast cancer patient treated with GcMAF, sonodynamic therapy and hormone therapy. *Anticancer. Res* 34(8), 4589-4593 (2014).
28. A.P. Association, Diagnostic and statistical manual of mental disorders. 4th edition (1994).
29. Enstrom AM, Van de Water JA, Ashwood P. Autoimmunity in autism. *Curr. Opin. Investig. Drugs* 10(5), 463-473 (2009).
30. Gesundheit B, Rosenzweig JP, Naor D, *et al.* Immunological and autoimmune considerations of Autism Spectrum Disorders. *J. Autoimmun* 44(), 1-7 (2013).
31. Bauman MD, Iosif AM, Ashwood P, *et al.* Maternal antibodies from mothers of children with autism alters brain growth and social behavior development in the rhesus monkey. *Transl. Psychiatry* 3(), e278 (2013).
32. Siniscalco D, Bradstreet JJ, Cirillo A, *et al.* The in vitro GcMAF effects on endocannabinoid system transcriptionomics, receptor formation, and cell activity of autism-derived macrophages. *J. Neuroinflammation* 11(), 78 (2014).
33. Korhonen H, Marnila P, Gill HS. Milk immunoglobulins and complement factors. *Br. J. Nutr* 84(Suppl 1), S75-80 (2000).
34. Burrin DG, Davis TA, Ebner S, *et al.* Colostrum enhances the nutritional stimulation of vital organ protein synthesis in neonatal pigs. *J. Nutr* 127(7), 1284-1289 (1997).
35. Koletzko B, Aggett PJ, Bindels JG, *et al.* Growth, development and differentiation: a functional food science approach. *Br. J. Nutr* 80(Suppl 1), S5-45 (1998).
36. Walker A. Breast milk as the gold standard for protective nutrients. *J. Pediatr* 156(2 Suppl), S3-7 (2010).
37. Tyler JW, Steevens BJ, Hostetler DE, *et al.* Colostral immunoglobulin concentrations in Holstein and Guernsey cows. *Am. J. Vet. Res* 60(9), 1136-1139 (1999).
38. Solomons NW. Modulation of the immune system and the response against pathogens with bovine colostrum concentrates. *Eur. J. Clin. Nutr* 56(Suppl 3), S24-28 (2002).
39. Snoeck V, Peters IR, Cox E. The IgA system: a comparison of structure and function in different species. *Vet. Res* 37(3), 455-467 (2006).
40. Basset C, Devauchelle V, Durand V, *et al.* Glycosylation of immunoglobulin A influences its receptor binding. *Scand. J. Immunol* 50(6), 572-579 (1999).
41. Inoue T, Iijima H, Tajiri M, *et al.* Deficiency of N-acetylgalactosamine in O-linked oligosaccharides of IgA is a novel biologic marker for Crohn's disease. *Inflamm. Bowel. Dis* 18(9), 1723-1734 (2012).
42. Uto Y, Kawai T, Sasaki T, *et al.* Degalactosylated/Desialylated Bovine Colostrum Induces Macrophage Phagocytic Activity Independently of Inflammatory Cytokine Production. *Anticancer. Res* 35(8), 4487-4492 (2015).
43. Oesser S, Adam M, Babel W, *et al.* Oral administration of (14)C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). *J. Nutr* 129(10), 1891-1895 (1999).
44. Cesta MF. Normal structure, function, and histology of mucosa-associated lymphoid tissue. *Toxicol. Pathol* 34(5), 599-608 (2006).
45. Brandtzaeg P, Kiyono H, Pabst R, *et al.* Terminology: nomenclature of mucosa-associated lymphoid tissue. *Mucosal. Immunol* 1(1), 31-37 (2008).
46. Fukuda F, Straus SE, Hickie I, *et al.* The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann. Intern. Med* 121(12), 953-959 (1994).
47. Klimas NG, Koneru AO. Chronic fatigue syndrome: inflammation, immune function, and neuroendocrine interactions. *Curr. Rheumatol. Rep* 9(6), 482-487 (2007).
48. Maes M, Mihaylova I, Kubera M, *et al.* Increased 8-hydroxy-deoxyguanosine, a

marker of oxidative damage to DNA, in major depression and myalgic encephalomyelitis/chronic fatigue syndrome. *Neuro. Endocrinol. Lett* 30(6), 715-722 (2009).

49. Maes M, Twisk FN. Why myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) may kill you: disorders in the inflammatory and oxidative and nitrosative stress (IO&NS) pathways may explain cardiovascular disorders in ME/CFS. *Neuro. Endocrinol. Lett* 30(6), 677-693 (2009).
50. Smits B, van den Heuvel L, Knoop H, *et al.* Mitochondrial enzymes discriminate between mitochondrial disorders and chronic fatigue syndrome. *Mitochondrion* 11(5), 735-738 (2011).
51. Morris G, Berk M, Galecki P, *et al.* The emerging role of autoimmunity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs). *Mol. Neurobiol* 49(2), 741-756 (2014).
52. Maes M, Twisk FN, Kubera M, *et al.* Evidence for inflammation and activation of cell-mediated immunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): increased interleukin-1, tumor necrosis factor-alpha, PMN-elastase, lysozyme and neopterin. *J. Affect. Disord* 136(3), 933-939 (2012).
53. Carlo-Stella N, Badulli C, De Silvestri A, *et al.* A first study of cytokine genomic polymorphisms in CFS: Positive association of TNF-857 and IFNgamma 874 rare alleles. *Clin. Exp. Rheumatol* 24(2), 179-182 (2006).
54. Morris G, Maes M. Increased nuclear factor-kappaB and loss of p53 are key mechanisms in Myalgic Encephalomyelitis/chronic fatigue syndrome (ME/CFS). *Med. Hypotheses* 79(5), 607-613 (2012).
55. Meeus M, Nijs J, McGregor N, *et al.* Unravelling intracellular immune dysfunctions in chronic fatigue syndrome: interactions between protein kinase R activity, RNase L cleavage and elastase activity, and their clinical relevance. *In. Vivo* 22(1), 115-121 (2008).
56. Inui T, Kubo K, Kuchiike D, *et al.* Oral Colostrum Macrophage-activating Factor for Serious Infection and Chronic Fatigue Syndrome: Three Case Reports. *Anticancer. Res* 35(8), 4545-4549 (2015).
57. Friese MA, Schattling B, Fugger L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nat. Rev. Neurol* 10(4), 225-238 (2014).
58. Friese MA, Fugger L. T cells and microglia as drivers of multiple sclerosis pathology. *Brain* 130(11), 2755-2757 (2007).
59. Milo R. Therapeutic strategies targeting B-cells in multiple sclerosis. *Autoimmun. Rev* 15(7), 714-718 (2016).
60. Romme Christensen J, Bornsen L, Ratzer R, *et al.* Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression. *PLoS. One* 8(3), e57820 (2013).
61. Inui T, Katsuura G, Kubo K, *et al.* Case Report: GcMAF Treatment in a Patient with Multiple Sclerosis. *Anticancer. Res* 36(7), 3771-3774 (2016).
62. Inui T, Amitani H, Kubo K, *et al.* Case Report: A Non-small Cell Lung Cancer Patient Treated with GcMAF, Sonodynamic Therapy and Tumor Treating Fields. *Anticancer. Res* 36(7), 3767-3770 (2016).