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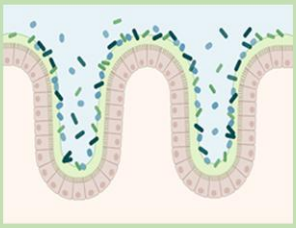

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1. COMPOSITION AND BIOACTIVE COMPONENTS OF CHLORELLA AND CGF

Chlorella species are unicellular green microalgae characterized by high macronutrient density and a rich spectrum of bioactive molecules. Across species and culture conditions, total protein typically constitutes 50–60% of dry biomass, with lipids and carbohydrates each contributing >10% [1], while additional fractions include pigments, vitamins, minerals, nucleic acids and polysaccharides [2] [3] [1] [4].

Proximate composition and major nutrient classes

Proteins are the dominant macronutrient in Chlorella, often exceeding 50% of dry weight in C. vulgaris and related species, and providing all essential amino acids in proportions comparable to or better than conventional plant proteins [3] [1] [5] [6]. Lipid contents are usually 10–20% of dry biomass, with a substantial share of polyunsaturated fatty acids such as linoleic and α-linolenic acids, which contribute to membrane fluidity and potential cardiometabolic benefits[1]. Carbohydrates, including storage polysaccharides and structural cell-wall components, also account for ≥10% of biomass and represent both an energy source and a source of functional fibers [2] [1] [4]. In addition, Chlorella supplies B-group vitamins, minerals, chlorophylls and carotenoids, adding nutritional value and antioxidant potential [2] [3] [1] [7] [8].

Chlorella Growth Factor (CGF) and protein/peptide fractions

CGF is obtained as a water-soluble fraction after cell disruption and extraction, and is consistently described as protein- and peptide-rich. Analyses of CGF from Chlorella cells report approximately 56% protein, 42–43% free amino acids, 6–7% nucleic acids, 4–5% polysaccharides and small amounts of vitamins [9]. In mixotrophically cultivated C. sorokiniana, CGF contained more than half of the cellular protein, with eight essential amino acids making up 26% of its amino-acid profile and a total essential amino acid content (13.7 g/100 g) slightly higher than soy meal [6]. Enzymatic hydrolysates of Chlorella proteins yield low-molecular-weight peptides enriched in hydrophobic and antioxidant amino acids, with high radical-scavenging, metal-chelating and angiotensin-converting enzyme (ACE)–inhibitory activities, making them attractive as functional ingredients [5] [10] [11]. In silico proteomic analyses of C. sorokiniana proteins further indicate a high frequency of encrypted sequences with predicted ACE-inhibitory, dipeptidyl peptidase-IV–inhibitory, antioxidant, antithrombotic and glucose-modulating activities [12].

Polysaccharides, pigments and minor bioactives

Polysaccharides are recognized as major active ingredients in Chlorella, with diverse structures and molecular weights conferring immunomodulatory, antioxidant, antihyperlipidemic, antitumor, neuroprotective and anti-asthmatic effects [4]. Structural features such as monosaccharide composition, glycosidic linkages and branching patterns are strongly associated with these bioactivities, and extraction/purification methods markedly influence yield and structure [2] [4]. Chlorella pigments, especially chlorophylls and carotenoids (e.g., lutein), function as potent antioxidants and photoprotective agents, relevant not only to nutrition but also to cosmetic and dermatological applications [2] [3] [13] [8]. Lutein and its geometric isomers can be efficiently recovered from CGF-extracted biomass, underscoring that CGF production is compatible with the subsequent valorization of lipid and pigment fractions [13].

Functional implications and formulation aspects

The combined presence of high-quality proteins/peptides, bioactive polysaccharides, polyunsaturated fatty acids, vitamins and pigments explains the wide biological activity profile of Chlorella ingredients, including antioxidant, anti-inflammatory, immunomodulatory, lipid-lowering, antidiabetic and detoxifying effects [3] [1] [5] [4] [10] [11]. CGF and Chlorella peptides have been incorporated into food, nutraceutical, cosmetic and biotechnological formulations; however, their practical use is constrained by instability and sensory issues such as bitterness, prompting development of microencapsulation systems to preserve activity and improve techno-functional properties in complex matrices [5] [14] [11]. Continued optimization of cultivation conditions and extraction/hydrolysis strategies is therefore central to tailoring Chlorella and CGF compositions for specific health and industrial applications [2] [5] [6] [15] [11].

Summary Table: Key Bioactive Components

Figure 1 Overview of major bioactive classes in Chlorella and CGF.

Component class	Typical role/bioactivity	Citations
Proteins/peptides (incl. CGF)	High-quality protein; antioxidant, ACE/DPP-IV inhibition, immunomodulation	[3] [1] [5] [12] [6] [10] [9] [11]
Polysaccharides	Immunomodulatory, antioxidant, lipid-lowering, antitumor, neuroprotective	[2] [3] [4]
Lipids (PUFA)	Membrane function, endothelial protection, performance support	[2] [1]
Pigments (chlorophyll, carotenoids, lutein)	Antioxidant, anti-aging, photoprotection	[2] [3] [8]
Vitamins, minerals, nucleic acids	Micronutrient supply; nucleotides for CGF bioactivity	[2] [3] [1] [9]

2. IMMUNOMODULATORY EFFECTS OF CHLORELLA AND CGF (EXPANDED)

Chlorella and CGF-type extracts influence key immune pathways (cytokines, Th1/Th2 balance, NK cells, macrophages, hematopoiesis). This section explains mechanisms in accessible terms and links them to practical relevance for health, disease prevention and product design.

Cytokines: Central "messengers" targeted by Chlorella

Cytokines are small proteins that immune cells use to "talk" to each other. They decide whether the body mounts a strong attack, calms inflammation, or builds long-term immunity.

2.1.1 Pro-inflammatory and anti-inflammatory cytokines

Hot-water extracts and polysaccharides from C. pyrenoidosa and C. vulgaris stimulate secretion of IL-1β, TNF-α, IL-6, IFN-γ, IL-12 and IL-10 in vitro and in vivo [16] [17] [3] [18].

Mechanistically:

- Polysaccharides (rich in rhamnose, glucose, galactose, etc.) from hot-water extracts bind Toll-like receptors (TLRs) on macrophages, triggering intracellular kinase cascades and leading to IL-1β release [16] [17]. IL-1β is a key early alarm signal during infection and tissue damage, recruiting immune cells and promoting fever [16] [17].
- Aqueous C. vulgaris stimulates human peripheral blood mononuclear cells (PBMCs) to secrete TNF-α and IL-6 at 1% (w/v). [18] TNF-α activates inflammation and helps kill infected or malignant cells, while IL-6 both promotes acute phase responses and, in later phases, tempers excessive TNF-α to keep inflammation in check. [18]
- Chlorella polysaccharides also boost IFN-γ and IL-2, which activate macrophages, NK cells and T cells. [3]
- Importantly, Chlorella increases IL-10, an anti-inflammatory cytokine that limits tissue damage and helps resolve inflammation, partly via modulation of the NF-κB pathway. [3]

Why this matters practically:

1. Infection resistance – Higher IL-1β, IFN-γ, IL-12 and TNF-α can improve early control of viruses and bacteria, especially in people with borderline immune function (ageing, chronic stress) [16] [17] [3].
2. Inflammation control – The parallel rise in IL-10 and IL-6 suggests Chlorella does not just "push the gas pedal" but also supports braking mechanisms, which is relevant for long-term use and for individuals with low-grade chronic inflammation. [3] [18]
3. Formulation choices – CGF-like hot-water extracts and polysaccharide-enriched fractions are the preparations most consistently associated with cytokine modulation [16] [17] [3]. For supplement or functional-food development aimed at immune support, these extracts are logical lead candidates.

Th1/Th2 Balance and Allergy Modulation

Helper T cells (Th cells) orchestrate immune responses:

- Th1: promotes cell-mediated immunity (viruses, intracellular bacteria, cancer cells) via IFN-γ, IL-2, IL-12.
- Th2: supports antibody (especially IgE) responses and is dominant in allergy (asthma, atopic dermatitis, allergic rhinitis) via IL-4, IL-5, IL-6, IL-13.

An imbalance toward Th2 is typical for allergic diseases. Chlorella extracts appear to rebalance from Th2 toward Th1.

2.1.2 Experimental evidence

Mice fed a diet containing 2% C. vulgaris hot-water extract for two weeks before antigen challenge showed [16] [17]:

- Suppressed IgE production and reduced IL-6 (Th2-associated).
- Increased IL-12 and IFN-γ mRNA, indicating enhanced Th1 responses.

These results indicate a shift toward Th1 dominance and suppression of Th2-mediated allergic responses [16] [17]. Mechanistically, TLR-activated antigen-presenting cells (e.g., macrophages, dendritic cells) exposed to Chlorella polysaccharides likely produce more IL-12, skewing naïve T cells into Th1 cells and dampening Th2 polarization. [16] [17] [3]

In humans, C. pyrenoidosa supplementation (200–400 mg/day) modulated responses to influenza vaccination, with age-dependent changes in specific immunity, again consistent with subtle reshaping of T-cell responses. [17]

Practical implications

1. Allergy support – For individuals with Th2-dominant conditions (e.g., environmental allergies), regular intake of CGF-like extracts might help reduce IgE-driven responses, especially if started before peak allergen seasons [16] [17]. This is not a replacement for pharmacotherapy but suggests a preventive adjunct strategy.
2. Vaccination – Modulation of Th1/Th2 balance can influence vaccine effectiveness. While human data are limited and variable, Chlorella might modestly enhance cell-mediated aspects of vaccine responses in some age groups [17].
3. Autoimmunity caution – In autoimmune diseases that are already Th1- or Th17-skewed, strong Th1-driving supplements may be a double-edged sword; careful clinical trials are required before generalized recommendations.

Natural Killer (NK) Cells: Front-line Defense Against Viruses and Tumors

NK cells are innate lymphocytes that kill virus-infected and cancer cells without prior sensitization. Their activity is heavily regulated by IFN-γ, IL-12 and IL-15.

2.1.3 Human clinical trial

A randomized, double-blind, placebo-controlled trial in healthy adults evaluated 5 g/day of C. vulgaris for 8 weeks [16] [17] [3]:

- Serum IFN-γ and IL-1β increased significantly versus baseline and placebo.
- IL-12 tended to increase.
- NK cell activity increased significantly in the Chlorella group, and NK activity correlated positively with IFN-γ and IL-1β [16] [3].

This indicates that short-term oral Chlorella can activate NK cells via cytokine up-regulation.

Mechanistically, ingested Chlorella (or CGF fractions) interacts with gut-associated lymphoid tissue (GALT) and immune cells, possibly via polysaccharide receptors and microbiota shifts, stimulating IL-12–IFN-γ axes that enhance NK cytotoxicity systemically. [16] [17] [3] [18]

Why NK enhancement is important

1. Antiviral defense – NK cells are crucial in early control of viral infections (influenza, herpesviruses, respiratory viruses). Higher NK activity can mean fewer symptomatic infections or shorter duration, especially in older or stressed individuals [16] [3].
2. Cancer immunosurveillance – NK cells patrol tissues and help eliminate emerging tumor cells. While direct anticancer evidence for Chlorella in humans is limited, the mechanistic link (↑NK activity, ↑IFN-γ) supports its exploration as a supportive measure in high-risk or post-therapy populations [3].
3. Practical use – The dose used in the RCT (5 g/day of whole C. vulgaris) is within common supplement ranges. For consumers, this suggests that commercially realistic intakes can have measurable immune effects within 1–2 months [16] [17] [3].

Macrophages, Innate Immunity and Antimicrobial Effects

Macrophages are “professional phagocytes” that engulf pathogens and dead cells. They also present antigens and shape downstream T-cell responses.

2.1.4 Macrophage activation by polysaccharides

The water-soluble polysaccharides from C. pyrenoidosa hot-water extracts, dominated by rhamnose and glucose, activate macrophage IL-1β production via TLR-dependent kinase pathways in mice [16] **3**. This mimics, though more gently than, the stimulation caused by microbial components such as bacterial lipopolysaccharide, priming the immune system to better respond to real infections.

In human PBMCs, aqueous C. vulgaris increased TNF-α and IL-6 secretion at 1% (w/v). [18] These cytokines are central to acute inflammation and help recruit neutrophils and monocytes to infection sites.

Practical implications

1. Enhanced first-line defense – Polysaccharide-rich CGF-like extracts may function similarly to mild, food-grade adjuvants, improving readiness of the innate immune system without the toxicity of pharmacological immunostimulants. [16] [17] [18]
2. Antimicrobial potential – By boosting IL-1β and TNF-α, Chlorella may contribute to better clearance of bacteria and fungi. This has implications not only for human supplements but also for animal feed, where Chlorella biomass and CGF are used to reduce infection rates and diminish the need for antibiotics. [7] [3].

3. Balance vs. over-activation – Because excessive TNF-α and IL-1β can contribute to chronic inflammation, long-term or high-dose use should be evaluated carefully in individuals with inflammatory or autoimmune conditions. The presence of IL-10-inducing activity is reassuring but does not replace clinical monitoring. [3] [18]

Hematopoiesis and Stress Protection

Beyond acute immune activation, Chlorella influences the production of immune cells in the bone marrow (hematopoiesis).

In mice subjected to physical and emotional stressors that normally suppress bone-marrow function, C. vulgaris supplementation preserved:

- Hematopoietic progenitor cells
- Mature myeloid and lymphoid populations
- Serum IL-1α and IL-6 at functional levels

This indicates protection against stress-induced myelosuppression and maintenance of immune capacity. [16]

Practical relevance

1. Chronic stress and immunity – Chronic psychological or physical stress (intense training, caregiving, shift work) can blunt immune responses. Chlorella's preservation of hematopoiesis suggests potential benefits for people under chronic stress, though direct human data are still needed [16].
2. Adjunct in immunosuppression – In theory, Chlorella might help support immune cell recovery after chemotherapy or other myelosuppressive interventions, but robust clinical evidence is lacking; any use here should remain investigational and supervised.
3. Animal production – In livestock, improved hematopoiesis and immune vigor can translate into better growth, survival and reduced veterinary costs when low levels of Chlorella/CGF are added to feed [7].

Gut–Immune Axis and Prebiotic Synergy

The gut microbiota is a major regulator of systemic immunity. Chlorella interacts both directly with immune cells and indirectly via microbiota.

In a functional-food model combining *C. vulgaris*, bovine colostrum and *Bifidobacterium animalis*:

- Chlorella promoted bifidobacterial growth (a beneficial genus).
- 1% (w/v) aqueous Chlorella significantly increased TNF-α and IL-6 production from human PBMCs in vitro. [18]
- In rats on a high-fat diet, the fermented colostrum–Chlorella combination lowered triglycerides and improved liver enzymes. [18]

Reviews also note that Chlorella can modulate gut microbiota and enhance immune responses, suggesting potential in autoimmune and inflammatory conditions, although clinical data are still sparse. [3]

Practical implications

1. Design of synbiotic products – Combining Chlorella with probiotics (e.g., Bifidobacteria) and prebiotic fibers may yield synergistic immunometabolic benefits (better lipid profiles, stronger innate immunity). [3] [18]
2. Barrier integrity and tolerance – By acting at the gut level, Chlorella-based products may help improve barrier function and immune tolerance, which are relevant for conditions like metabolic syndrome and low-grade gut inflammation, but this remains a research frontier. [3]
3. Dosing considerations – In vitro, a lower Chlorella concentration (1% w/v) elicited stronger cytokine responses than 3%, suggesting a non-linear dose–response and supporting moderate dosing for immune modulation. [18]

Integrative Practical View: How to Leverage These Effects

From the mechanistic and experimental data, several practical guidelines can be derived for human use and product development:

- Target endpoints
    - General immune support, especially in healthy or mildly immunocompromised adults: use whole C. vulgaris (2–5 g/day) or standardized CGF extracts focusing on polysaccharides and nucleotides [16] [17] [3].
    - Allergy-prone individuals (Th2-biased): emphasize CGF-like hot-water extracts, started weeks before allergy season to encourage Th1 rebalancing [16] [17].
    - Gut-focused functional foods: combine Chlorella powder with probiotics and fermentable substrates (colostrum, dairy or plant matrices). [18]
- Form and composition
    - Whole biomass (powder, tablets) delivers a broad spectrum (proteins, pigments, vitamins, polysaccharides) and has proven NK-enhancing effects at 5 g/day. [16] [17] [3]
    - Hot-water or CGF-type extracts concentrate nucleotides, peptides and polysaccharides, which are likely main drivers of cytokine and Th1/NK modulation. [16] [17] [3]
    - Standardization (e.g., polysaccharide content, UV–vis/LC-MS fingerprints) should be pursued for reproducible immune effects. [19] [13]
- Populations needing caution
    - Autoimmune disease, organ transplants, or patients on immunosuppressants: immunostimulation could, in theory, interfere with therapy; clinical guidance is required.
    - People with chronic inflammatory conditions: potential benefits (IL-10, regulatory effects) must be weighed against pro-inflammatory cytokine induction.
- Evidence gaps
    - Most detailed mechanistic data are from animal and in vitro studies; high-quality RCTs in specific clinical populations (allergy, recurrent infections, autoimmune disease, oncology) are limited. [16] [17] [3] [18]
    - Comparisons between different species (C. vulgaris vs. C. pyrenoidosa), extraction methods and doses are scarce, making it difficult to define “optimal” preparations. [16] [17] [3] [20].

Summary

Chlorella and CGF-type preparations modulate immunity on multiple levels:

- Cytokines: activate IL-1β, TNF-α, IL-6, IFN-γ, IL-12 and IL-10, orchestrating a balanced inflammatory and regulatory response.
- Th1/Th2 balance: shift responses toward Th1, lowering IgE and potentially attenuating Th2-driven allergy.
- NK cells: increase NK cell activity and related cytokines in humans at nutritionally realistic doses.
- Macrophages and innate defense: polysaccharides act via TLR pathways to prime antimicrobial responses.
- Hematopoiesis and stress protection: support bone-marrow function and immune resilience under stress in animal models.
- Gut–immune axis: interact with microbiota and synergize with probiotics and colostrum in functional foods.

These converging immunomodulatory actions, combined with generally good tolerability, explain why Chlorella has become a prominent natural immunonutrient for both human and animal applications. Further standardized clinical research is needed to translate the mechanistic promise into precise, indication-specific recommendations.

3. EFFECTS OF CHLORELLA/CGF ON GUT HEALTH & MICROBIOME

Chlorella interacts with the gut on several levels: microbiota composition, production of microbial metabolites (especially short-chain fatty acids, SCFAs), barrier integrity, and gut–immune crosstalk. Together these effects help explain both local digestive benefits and wider systemic effects.

Why the Gut Microbiome Matters

The gut microbiota is a dense ecosystem (over 1000 species) that contributes to digestion, vitamin synthesis, immune education, barrier integrity and metabolism. [21] [22] [23] Healthy microbiota are diverse, dominated by Firmicutes and Bacteroidetes, and produce SCFAs through fermentation of non-digestible substrates like dietary fibers and prebiotics. [21], [22] [23] [24]

Disturbances (dysbiosis) are linked with obesity, inflammatory bowel disease (IBD), allergies, autoimmune disease and even neuropsychiatric disorders via the microbiota–gut–brain axis [21] [22] [25] [26]. Because of this, ingredients that can shift the microbiome toward SCFA-producing, barrier-supporting taxa are of high interest as functional foods.

Chlorella behaves partly like a prebiotic-type substrate: it is not fully digested in the upper gut, reaches the colon, and is then fermented by microbes with measurable effects on both community structure and metabolites [27] [28] [29].

Chlorella as a Substrate for SCFA Production

SCFAs (acetate, propionate and butyrate) are key metabolites of gut bacteria. They:

- Provide energy for colonocytes (butyrate)
- Strengthen tight junctions and barrier integrity
- Modulate immune cells and promote regulatory T cells (Tregs)
- Influence glucose and lipid metabolism and systemic inflammation [27] [30] [22] [26] [23] [31] [32]

3.1.1 In vitro digestion–fermentation of C. vulgaris

Simulated gastrointestinal digestion followed by colonic fermentation of C. vulgaris showed that a significant fraction of Chlorella material reaches the colon (bioaccessibility ≈ 0.24 g/g) and is fermented by microbiota [28]. This fermentation:

- Significantly increased acetate, propionate and butyrate compared with controls [27] [28]
- Was associated with higher abundance of SCFA-linked genera such as Faecalibacterium, Roseburia, Dialister, Megasphaera, Veillonella and Bifidobacterium [28]

A similar in vitro study with C. vulgaris digests confirmed increased production of acetic, propionic, butyric and isobutyric acids after 48 h fermentation, and concluded that Chlorella digests are a promising ingredient for good intestinal health and balanced colonic microbiota [27].

Pressurized-liquid extracts of microalgae (including Chlorella) also promoted SCFA release (acetic, butanoic, propanoic) during colonic fermentation, reinforcing that microalgal biomolecules are fermentable substrates for SCFA production [33].

3.1.2 C. pyrenoidosa fermentation in human gut models

An in vitro continuous gut model inoculated with microbiota from a healthy and a coeliac donor showed that C. pyrenoidosa:

- Increased butyrate and propionate from baseline in the healthy donor
- Altered pathways related to SCFA production in both healthy and coeliac microbiota [29]

These findings position Chlorella as a SCFA-enhancing ingredient, similar in principle to classic prebiotic fibers [21] [23] [31][24].

Practical significance

- Increased butyrate supports colonocyte energy metabolism, tight junctions and mucosal healing, and is protective against colitis and colorectal carcinogenesis [27] [30] [22] [26] [23] [31].
- Acetate and propionate contribute to appetite regulation, lipid and glucose metabolism, and systemic anti-inflammatory effects via gut hormones and direct receptor signaling [22] [26] [23] [31].
- For product developers, this means Chlorella (particularly minimally processed biomass or extracts retaining complex carbohydrates) can be positioned as a SCFA-supporting functional ingredient.

Microbiota Composition: Promoting Beneficial Taxa, Reducing Potential Pathogens

3.1.3 Human-relevant in vitro models

C. vulgaris digests shifted in vitro faecal communities by:

- Decreasing potentially dysbiosis-associated taxa such as certain Clostridium, Enterobacteriaceae and Bacteroides groups considered markers of intestinal imbalance [27]
- Enriching health-related taxa such as Bifidobacterium and Lactobacillus, and SCFA-producing genera (Faecalibacterium, Roseburia, Butyricimonas, Odoribacter, Veillonella, etc.) [27] [33] [28]

Pressurized microalgal extracts inhibited growth of pathogenic bacteria in vitro while enhancing growth of beneficial Lactobacillus and Bifidobacterium strains[33]. This suggests selective support of desirable microbes and suppression of opportunists.

An in vitro model with C. pyrenoidosa found increases in Prevotella and Bifidobacterium (among others) in healthy microbiota, and decreases in Enterobacteriaceae in coeliac microbiota, again consistent with a shift away from potentially harmful proteobacteria [29].

3.1.4 Animal models

In weaned piglets fed 5% C. vulgaris, performance was maintained but gut health markers improved: villus height in the duodenum increased and specific beneficial taxa such as Colidextribacter,

Oscillospira and Lactobacillus were more abundant, indicating a “healthier” microbiota and mucosa despite slightly reduced overall nutrient digestibility [34].

In broiler chickens, dietary C. vulgaris increased alpha diversity (Chao1 and observed features) in the cecal microbiome and enriched several genera considered beneficial; at the same time, intestinal barrier markers and CD4+ T cells increased, linking microbiota shifts, barrier support and mucosal immunity [35].

A broiler study combining graded C. vulgaris (up to 1%) with a probiotic found that by day 42, Bifidobacterium spp. appeared only in birds receiving 1% microalgae plus probiotics, suggesting that Chlorella can provide substrates or conditions that favor colonization by classic probiotic genera [36].

Practical significance

- The repeated enrichment of Bifidobacterium, Lactobacillus and butyrate-producers points toward prebiotic-like properties of Chlorella [27] [33] [34] [28] [29].
- Reduction of Enterobacteriaceae and other dysbiosis markers is particularly relevant for populations prone to intestinal inflammation or infection.
- In animal nutrition, low-dose Chlorella in feed can support gut health and reduce reliance on antibiotics by improving barrier and microbial resilience [35] [36] [34].

Gut Barrier Integrity and Mucosal Morphology

A critical function of the microbiota–diet–host interaction is maintaining an intact intestinal barrier, preventing translocation of pathogens and toxins and limiting systemic inflammation.

3.1.5 Barrier markers and morphology

In broilers, C. vulgaris supplementation increased expression of occludin (a tight-junction protein) and avian β-defensin-5, and increased villus height in the duodenum, indicating improved barrier function and absorptive surface [35]. Piglets receiving 5% C. vulgaris also showed increased villus height without impaired growth, suggesting adaptive mucosal development in response to slightly lower digestibility [34].

SCFAs—particularly butyrate—directly promote tight junction assembly, mucin production and epithelial differentiation, and serve as the main fuel for colonocytes [30] 7 [26] [23] [31]. Since Chlorella consistently increases SCFA production in fermentation models, part of its barrier-supportive effect is likely mediated indirectly via these metabolites [27] [33] [28] [29].

Practical significance

- Stronger barrier function means reduced “leaky gut”, lower LPS translocation and less systemic low-grade inflammation, which is relevant for cardiometabolic health, autoimmunity and even mental health [21] [30] [22] [25] [26] [23].
- For people with sensitive digestion or early-stage inflammatory bowel conditions, barrier-supporting foods like Chlorella (alongside classic fibers) could form part of a diet aimed at mucosal healing and resilience, though direct clinical evidence in humans is still emerging.

Gut–Immune Crosstalk: Regulatory T Cells and Colitis Models

A key mechanistic link between microbiota changes and systemic effects is immune modulation via SCFAs and microbial antigens.

3.1.6 C. vulgaris, Tregs and SCFAs in colitis

In mice, dietary C. vulgaris altered gut microbiota and increased SCFA production under homeostatic conditions. When these mice were subjected to DSS-induced colitis, Chlorella intervention:

- Alleviated clinical and histological signs of colitis
- Increased regulatory T cell (Treg) levels in the gut, indicating promotion of immune tolerance [37]

Given that butyrate and propionate are known to induce Tregs through G-protein-coupled receptors and histone deacetylase inhibition [30] [26] [23] [31], the SCFA increase observed with Chlorella is a plausible mechanistic bridge from microbiota modulation to Treg-mediated anti-inflammatory effects.

3.1.7 SCFA-centered perspectives

Reviews of SCFA biology emphasize that:

- Butyrate, acetate and propionate regulate barrier function, effector and regulatory T cells, B cells, phagocytes and autoantibody production, thereby influencing autoimmune and inflammatory diseases [30] [26] [23] [31] [32].
- Adequate SCFA levels correlate with lower risk or improved outcomes in IBD, metabolic disease and some extra-intestinal conditions [30] [26] [23] [31] [32].

By acting as a fermentable substrate that boosts SCFAs and enriches SCFA-producing taxa, Chlorella taps into this established protective pathway.

Practical significance

- For IBD-like conditions and colitis models, Chlorella shows proof-of-concept in animals for symptom alleviation via Treg expansion and SCFA-linked microbiota shifts [37].
- In humans, this supports the rationale for testing Chlorella as part of multi-component dietary strategies alongside fibers and probiotics to promote immune tolerance and dampen gut inflammation, but rigorous clinical trials are needed before therapeutic claims.

Human Studies: Individual Response and Metabolome Changes

A randomized, double-blind, placebo-controlled crossover trial in 40 adults with constipation assessed C. vulgaris supplementation and included gut microbiome and metabolome analyses [38]:

- No significant average improvement in defecation frequency (primary outcome) versus placebo.

- Exploratory analysis showed Chlorella increased several fecal dicarboxylic acids and that individuals with low baseline propionate experienced increases in fecal propionate after intake [38].
- Baseline defecation frequency modulated blood folate responses, indicating that individual gut environments shape the nutritional effect of Chlorella [38].

These results highlight inter-individual variability: Chlorella may strongly benefit people with certain baseline microbiota/metabolite profiles (e.g., low propionate producers) while having modest average effects.

Practical significance

- Precision nutrition: Chlorella's gut effects are not "one-size-fits-all." Baseline microbiota composition and metabolite levels likely determine who responds in terms of SCFA increase and clinical benefits [38].
- For practitioners, this suggests stratifying or at least monitoring gut markers when using Chlorella as an adjunct in gut-focused interventions.

Chlorella in the Broader Context of Pre-, Pro- and Postbiotics

Reviews on prebiotics, probiotics and postbiotics emphasize that:

- Prebiotics are non-digestible ingredients that selectively stimulate beneficial microbes, leading to SCFA production and improved immunity, metabolism and mental health [39] [21] [25] [23] [32] [24].
- Postbiotics such as SCFAs themselves and antimicrobial peptides can directly regulate pathogen growth, barrier function and immune responses [39] [30] [25] [26] [23] [32].

Chlorella occupies an interesting niche:

- Its indigestible carbohydrates and complex biomolecules behave prebiotic-like, fostering SCFA producers and beneficial genera [27] [33] [34] [28] [29].
- The resulting SCFA profile and other microbial metabolites act as postbiotic mediators of health benefits [27] [30] [22] [26] [23] [31] [32].
- When combined with probiotics (e.g., Bifidobacteria), Chlorella can function within synbiotic formulations, supporting colonization and activity of the probiotic strains [36] [33] [24].

Practical Applications and Design Considerations

For product development and clinical application:

- Form and processing
    - Whole biomass and gentle processing that preserve fibrous cell walls and polysaccharides are likely best for microbiota modulation and SCFA production [27] [33] [28] [29].
    - Highly purified extracts with minimal fermentable material may have weaker microbiome-targeted effects, though they can retain other bioactivities.

- Target uses
    - General gut health: modest daily doses of C. vulgaris or C. pyrenoidosa as part of a fiber-rich diet to support SCFA production, barrier integrity and microbial diversity.
    - Metabolic and inflammatory support: inclusion in multi-ingredient formulas (with fibers, probiotics) aiming to enhance butyrate and propionate producers and Treg-supportive SCFA profiles [37] [30] [26] [23] [31].
    - Animal feeds: low-level inclusion to improve gut morphology, microbial balance and performance while potentially reducing need for antibiotics [35] [36] [34].
- Caveats and gaps
    - Many data come from in vitro systems and animal models; human trials are few and show heterogeneous outcomes [27] [37] [33] [38] [28] [29].
    - Dose–response relationships and optimal species/strains of Chlorella for gut endpoints remain unclear.
    - Individual microbiome differences strongly influence response, pointing toward future personalized Chlorella use rather than uniform recommendations [38].

Summary

Chlorella and CGF-type preparations affect gut health through:

- Serving as fermentable substrates that increase SCFA production (acetate, propionate, butyrate)
- Enriching beneficial and butyrogenic taxa (Faecalibacterium, Roseburia, Bifidobacterium, Lactobacillus) and reducing potentially harmful groups like Enterobacteriaceae
- Supporting mucosal barrier integrity and villus morphology, partly through SCFA-mediated effects and direct modulation of tight junction and defensin expression
- Promoting regulatory immune responses (Tregs) and alleviating colitis in mice, linking microbiota changes to anti-inflammatory outcomes
- Exhibiting inter-individual variability in humans, pointing toward personalized applications

Positioned within the broader prebiotic–postbiotic framework, Chlorella emerges as a versatile gut-modulating immunonutrient, with promising but still evolving evidence base for both human health and animal production contexts.

4. OTHER HEALTH BENEFITS OF CHLORELLA GROWTH FACTOR (CGF)

Beyond immunity and gut health, CGF shows antioxidant, metabolic, tissue-regenerative and protective effects that are relevant for both human health and biotechnology.

Composition and General Functional Profile

Enzymatically extracted CGF typically contains ~55–60% protein, ~40% amino acids, 5–7% nucleic acids, and minor polysaccharides and vitamins [40]. This dense mix of peptides, nucleotides and micronutrients underlies its:

- Growth-promoting and wound-healing effects
- Immunostimulatory and adaptogenic properties
- **Potential use as a functional food ingredient** [40] [41] [7]

Metabolic & Cardiovascular Support

Although most clinical data are on whole Chlorella, many mechanisms are attributable to CGF-rich water extracts.

- Meta-analyses and reviews on Chlorella supplementation show reductions in total and LDL cholesterol, systolic/diastolic blood pressure, and fasting glucose, with no consistent effect on triglycerides or HDL [16] [42].
- Animal and human work suggests Chlorella (and its extracts) can lower serum lipids and improve atherogenic profiles, partly through reduced intestinal lipid absorption and changes in serum carotenoids [16].
- In broilers, dietary CGF (0.05–0.15%) reduced serum total lipids and increased IgG and IgM, supporting a dual lipid-modulating and immunotropic role[41].
- In rats on a high-fat diet, colostrum fermented with Chlorella and Bifidobacterium lowered triglycerides, VLDL and liver enzymes, indicating hepatoprotective, triglyceride-lowering synergy [18] [43].

Practical angle: CGF-containing Chlorella products can be positioned as adjuncts for metabolic syndrome and cardiovascular risk, especially when combined with probiotics or colostrum [42] [16] [43] [18]. Doses in trials typically correspond to several grams/day of whole Chlorella (CGF fraction not always standardized).

Antioxidant & Cytoprotective Effects

Chlorella supplementation enhances antioxidant defenses and mitigates toxic injury in multiple models:

- Review data highlight Chlorella's antioxidant activities alongside antidiabetic, antihypertensive and antihyperlipidemic effects, likely via synergistic nutrients and antioxidants [16].

- In Nile tilapia chronically exposed to chlorpyrifos, Chlorella (1–3% of diet) normalized expression of heat-shock proteins and antioxidant enzymes (GPx, GSS, GSR), and modulated pro-inflammatory cytokines, reducing pesticide-induced oxidative and immune stress [44].
- In mice exposed to carcinogenic benzo(a)pyrene, a CGF concentrate normalized phagocytic function and leukocyte subsets and alleviated benzo(a)pyrene-induced immunotoxicity, acting as an adaptogen and cellular-protection factor [45].

Why this matters: In real life, chronic low-grade exposure to pollutants, pesticides and oxidative stressors is common. CGF-rich Chlorella may help buffer antioxidant systems and immune defenses, potentially reducing damage from such exposures [16] [45] [44].

Skin Health, Wound Healing and Anti-Aging

CGF shows direct relevance to skin regeneration and cosmetic applications:

- In human keratinocyte (HaCaT) cultures, CGF increased metabolic activity (extracellular acidification rate) in a dose-dependent manner, mimicking or exceeding EGF-like growth stimulation via epidermal growth factor receptor signaling [46].
- CGF stimulated proliferation of human epidermal keratinocytes, suggesting enhanced epidermal renewal and wound repair potential [46].
- In human fibroblast (Hu02) cells, optimally extracted CGF (ultrasonication + enzymatic hydrolysis) increased type I collagen mRNA ~3.1-fold and type III collagen ~1.1-fold, indicating stimulation of dermal collagen synthesis, a key anti-aging mechanism [47].

Practical angle:

- CGF-standardized extracts are promising active ingredients for topical anti-aging, wound-healing and barrier-support products, and potentially for oral “nutricosmetic” formulations.
- Mechanistically, they may combine growth-factor-like signaling (EGFR) with antioxidant and nutrient support, promoting both epidermal turnover and dermal matrix integrity [47] [46].

Growth, Performance and Reproductive Support (Animal Models)

In animal nutrition, CGF and Chlorella biomass confer several performance-related benefits:

- Low-dose Chlorella biomass improves growth, feed efficiency and reproduction across species; these effects are partially attributed to pigments, vitamins and CGF [7].
- In broilers, CGF did not match antibiotics or whole dried Chlorella for growth promotion, but still enhanced humoral immunity (higher IgG, IgM) and lowered serum lipids, supporting its role as an antibiotic alternative component [41].
- Reviews emphasize Chlorella’s use as a carrier for organically bound selenium and iodine, improving thyroid function and enriching animal products (eggs, meat) with these trace elements for human consumption [7].

Implication for humans: Animal data strengthen the concept of CGF as a general growth and resilience factor, which aligns with earlier observations of improved growth and healing in mammals and humans receiving CGF-rich preparations [41] [7].

Biotechnological & Regenerative Medicine Applications

CGF is emerging as a tool in cell culture, bioprocessing and tissue engineering:

- Aqueous CGF from C. vulgaris enhanced proliferation of CHO cells and mesenchymal stem cells under reduced-serum conditions, in both 2D and 3D culture, for up to 21 days [19].
- CGF supplementation increased therapeutic protein expression (GFP) ~5–6-fold in transfected CHO cells, suggesting activation of protein-expression pathways and improved bioproduction yields [19].
- Mesenchymal stem cells maintained stem-like phenotype (CD73+/CD90+/CD105+, CD45–) across passages with CGF, supporting its safety as a growth additive in tissue-engineering applications [19].

Practical angle:

- CGF can function as a serum-replacement or media additive in industrial cell culture, potentially lowering costs and animal-derived components while enhancing yields [19].
- Its growth-supportive yet phenotype-preserving effects make it attractive for regenerative medicine and TERM (tissue engineering and regenerative medicine) workflows.

Stress Resistance & Adaptogenic Properties

Metabolomics-based evaluation in mice indicates that Chlorella and CGF shift systemic metabolites related to energy, amino acid and antioxidant pathways, consistent with improved resistance to exercise-induced stress and metabolic syndrome **2**. While detailed pathways are still being mapped, this supports historical positioning of CGF as an adaptogen that improves resilience to physical and metabolic challenges [40] [41] [7].

Conclusion

Beyond immunomodulation and gut health, CGF contributes to:

- Cardio-metabolic support (better lipids, blood pressure, glucose)
- Antioxidant and cytoprotective defense, including against toxins
- Skin regeneration and anti-aging via keratinocyte proliferation and collagen synthesis
- Growth, performance and reproductive support in animals, with implications for human nutrition
- Biotechnological uses as a serum-replacement and protein-production enhancer in cell culture

Most human-level clinical evidence still comes from whole Chlorella; however, mechanistic and experimental data increasingly justify CGF-standardized products and biomedical applications.

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