Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health?

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Purpose of review

High-fiber diets have been shown to reduce plasma concentrations of inflammation markers. Increased production of fermentation-derived short-chain fatty acids (SCFAs) is one of the factors that could exert these positive effects. This review examines the effects of SCFAs on immune cells and discusses the relevance of their effects on systemic inflammation, as frequently seen in obesity.

Recent findings

SCFAs have been shown to reduce chemotaxis and cell adhesion; this effect is dependent on type and concentration of SCFA. In spite of conflicting results, especially butyrate seems to have an anti-inflammatory effect, mediated by signaling pathways like nuclear factor-κB and inhibition of histone deacetylase. The discrepancies in the results could be explained by differences in cell types used and their proliferative and differentiation status.

Summary

SCFAs show anti-inflammatory effects and seem to have the potency to prevent infiltration of immune cells from the bloodstream in, for example, the adipose tissue. In addition, their ability to inhibit the proliferation and activation of T cells and to prevent adhesion of antigen-presenting cells could be important as it recently has been shown that obesity-associated inflammation might be antigen-dependent. More studies with concentrations in micromolar range are needed to approach more physiological concentrations.

Keywords

dietary fiber, low-grade inflammation, obesity, prebiotics, short-chain fatty acids

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Introduction

The last decades it has become clear that overnutrition has impact on the immune system. Chronic overnutrition leads to accumulation of fat in adipose tissue, which subsequently can become infiltrated with immune cells. This gives rise to a mild and sustained increase in immune mediators in the systemic circulation, for example, the acute-phase response marker C-reactive protein (CRP) and its major regulators interleukin (IL)-6 and tumor necrosis factor (TNF)-α [1]. In addition, repeated exposure to increased concentrations of proinflammatory cytokines in the postprandial period after high-fat meals or rapidly digestible carbohydrates has recently postulated to be involved in the development of low-grade inflammation in susceptible persons [2,3]. Chronic low-grade inflammation is associated with an increased risk of, among others, insulin resistance, diabetes type 2, and atherosclerosis [4–6]. For this reason, strategies to suppress low-grade inflammation as a preventive measure for these chronic diseases are relevant to investigate. Food and food-derived substances receive increasing attention as potential factors that can modulate cells or cell functions that play a role in immunological processes. Dietary fiber intake has been shown in prospective studies to be inversely related to plasma concentrations of CRP [7–9] and the proinflammatory cytokine IL-6 [10]. In a human study on healthy elderly, immunomodulatory effects of the prebioticum B-galactooligosaccharides (B-GOSs) were demonstrated [11]. B-GOS significantly increased the production of the anti-inflammatory cytokine IL-10 and significantly reduced production of IL-6, IL-1β, and TNF-α [11]. Another intervention study on elderly with fructooligosaccharides resulted in decreased IL-6 mRNA expression in peripheral blood monocytes [12]. In healthy young individuals, an evening meal rich in nondigestible carbohydrate prevented the glucose-induced postprandial rise in plasma IL-6 and TNF-α concentrations [13•]. One of the proposed factors that could explain these effects is the increase in short-chain fatty acids (SCFAs) acetate, propionate, and butyrate, which are produced by the colonic microbiota when dietary fiber is fermented [14–16]. SCFAs are rapidly absorbed from the colonic lumen and partly metabolized by colonic epithelial cells. A proportion also enters the portal and peripheral circulation. In six

nonfasted sudden death victims, portal concentrations of acetate, propionate, and butyrate lay between 108 and 404 μmol/l, 17 and 194 μmol/l, and 14 and 64 μmol/l, respectively and peripheral concentrations between 19 and 146 μmol/l, 1 and 13 μmol/l, and 1–12 μmol/l, respectively [17].

SCFAs, and especially butyrate, have for long been the center of interest for modulating inflammatory responses in the colonic epithelial cells and results of these studies indicate beneficial effects. Therefore, it seems worthwhile to explore whether SCFAs could also affect systemic inflammation. This is especially interesting because, as discussed below, recent studies suggest that obesity-induced inflammation is partly antigen-dependent. Information about, for instance, the capacity of SCFAs to reduce activation of T cells by monocyte-presented antigens is, thus, highly relevant in this context. This review summarizes the results of studies investigating the effects of SCFAs on immune cells and discusses the relevance of these observations for the purported effects on systemic inflammation and consequently for the development of the metabolic syndrome.

Systemic low-grade inflammation

Inflammation of adipose tissue as well as circulating mononuclear cells that are in a proinflammatory state is proposed to contribute to systemic inflammation in obesity. An increase in the proinflammatory transcription factor nuclear factor-κB (NF-κB) in the nucleus together with a decrease in its inhibitors IκB-α and/or IκB-β is reflecting inflammation at the cellular level. These characteristics were present in peripheral blood mononuclear cells (PBMCs: lymphocytes, monocytes, and macrophages) of obese persons and increased NF-κB activity correlated positively with mRNA levels of IL-6 [18]. Postprandial, an inflammatory response to meals with a high content of fat (30–50 g) and/or rapidly digestible carbohydrates (e.g., sugars, white flour) has been found in PBMCs and plasma even in normal-weight individuals [19,20], which was aggravated in obese persons [21]. Repeated exposure to postprandial inflammatory responses is suggested to contribute to systemic inflammation and type 2 diabetes [2,3] as well as to vascular disease [2,22]. Very recently, postprandial activation of NF-κB and an increase in IL-6 mRNA has been shown in visceral adipose tissue after a high-fat meal (60% of the total energy from lipids) in rats [23].

However, generally, low-grade inflammation is thought to originate from the adipose tissue, though the initiating factors are still a matter of discussion. Hypertrophied adipocytes secrete abnormally high amounts of chemoattractants, such as the monocyte chemoattractant protein-1 (MCP-1), which causes accumulation of macrophages in adipose tissue [24]. These adipose tissue macrophages are considered the major source of proinflammatory cytokines [25]. More recently, it became clear that obese adipose tissue also contains various subsets of lymphocytes [26,27•,28•,29,30•]. The proinflammatory T-cell populations (cytotoxic T-cells and T_H1 cells) were increased in murine [27•,28•] and human obese adipose tissue [26,29]. Regulatory T (T_{reg}) cells, which contribute to the homeostasis of the immune system by suppressing immune response of other cells, have been demonstrated to be significantly decreased in obese mice [27•,28•] but not in obese humans [29]. However, in humans, a relative lack of T_H2 cells in obese visceral adipose tissue has been proposed to lead to an unfavorable T_H1/T_H2 balance, which has been shown to positively correlate with plasma CRP, IL-6, and TNF-α concentrations [29]. Therefore, the failure of T_H2 cells to counterbalance the effect of proinflammatory T_H1 cells might contribute to inflammation. Notably, however, the theory of a mandatory T_H1/T_H2 balance for immune homeostasis does not hold for all organ systems. For example, in the mucosal immune compartment, T_H2 responses are only observed under helminth infections and not under other circumstances. The balance in the mucosal site is considered to be dependent on T_H1 and T_H17 responses and kept in balance by T_{reg} cells [31]. It cannot be excluded that similar systems are responsible for immune homeostasis in adipose tissue.

In addition, changes in T-cell receptor diversity and rearrangements are reported in adipose tissue lymphocytes, suggesting that obesity-induced inflammation might be partly antigen-dependent [27•,28•,30•].

Receptors for short-chain fatty acids

The effects of SCFAs on immune cells might be mediated by G protein-coupled receptors (GPRs). The major SCFA receptors are GPR41 and GPR43, which are both expressed on immune cells [32,33]. GPR43 is highly expressed in polymorphonuclear cells (PMN, e.g., neutrophils) and at lower levels in PBMCs and purified monocytes. GPR41 is similarly expressed in PBMCs but not in PMNs, monocytes, and dendritic cells [33]. Both receptors are equally expressed in bone marrow and spleen. GPR41 and GPR43 were also found to be expressed in human adipose tissue [32,33]. Brown *et al.* [32] showed that GPR41 levels in adipocytes are low, whereas they are high in blood vessel endothelial cells, especially in adipose tissue. Furthermore, higher expression of both receptors has been shown in subcutaneous tissue than that in omental tissue of obese women [34]. GPR41 and GPR43 are activated by SCFAs with a different rank order of potency. For GPR41, this rank order was propionate > butyrate > acetate with acetate 100-fold less potent as compared to propionate and for

GPR43, it was propionate > acetate = butyrate [33]. The possible immune-modulatory functions of SCFAs are highlighted by a recent study on GPR43−/− mice [35••]. These mice exhibit aggravated inflammation, related to increased production of inflammatory mediators and increased immune cell recruitment.

Effect of short-chain fatty acid on chemotaxis and adhesion of immune cells

Effects of SCFAs on chemotaxis and adhesion of immune cells are interesting in context of the role that recruitment and accumulation of monocytes play in adipose tissue as well as in smooth muscle cells in atherosclerotic lesions.

Chemotaxis

Concentrations of 0.1 and 1 mmol/l but not of 10 mmol/l of acetate and propionate have been shown to increase the chemotactic response of neutrophils [33]. In accordance with these results, Sina *et al.* [36] demonstrated increased chemotaxis of isolated mice neutrophilic granulocytes after incubation with 0.1 mmol/l propionate and butyrate. The noneffectiveness of high concentrations was confirmed in a study that showed that incubation with 5 mmol/l butyrate did not affect migration of human neutrophils [37]. In contrast, chemotactic responses of mice neutrophils after 4 h pretreatment with 25 mmol/l acetate, 12 mmol/l propionate, and 4, 8, 12 mmol/l of butyrate were increased in the study of Vinolo *et al.* [38•]. This was accompanied by an increased expression of the adhesion molecule L-selectin but not β2 integrin on the surface of neutrophils [38•].

These results indicate a possible stimulatory effect of SCFA on chemotaxis of neutrophils in different concentrations in the absence of a chemotactic stimulator. Further, spontaneous migration of human and rat neutrophils and migration induced by the bacterial peptide formyl-Met-Leu-Phe (fMLP) were neither affected by 1 mmol/l of acetate and propionate [33] nor by higher concentrations of acetate, propionate, and butyrate [38•].

In contrast, preincubation of murine macrophages (RAW 264.7) and rat primary macrophages with 2 mmol/l butyrate was recently demonstrated to reduce lipopolysaccharide (LPS)-induced migration [39••]. The effect of SCFAs on MCP-1 production of human monocytes and PBMCs was assessed by Cox *et al.* [40•]. Overnight incubation with acetate, propionate, and butyrate (0.2, 2, and 20 mmol/l) inhibited constitutive MCP-1 production in a concentration-dependent manner either in absence or presence of LPS, with the strongest effect for butyrate.

The foregoing data illustrate that SCFA can influence chemotaxis of immune cells but that its effect depends on the type and concentration of the SCFA as well as on the type of species and immune cell type. Butyrate seems to be the most potent inhibiting factor for chemotactic effects on human monocytes. Probably, more effects are present, but remain to be identified.

Adhesion molecules

Differential effects of SCFA on expression of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were found. A 24-h incubation of human umbilical vascular endothelial cells (HUVECs) with 2 and 4 mmol/l of butyrate increased ICAM-1 expression without affecting VCAM-1 expression [41], which was confirmed in the study of Miller *et al.* [42] with 2.5–5 mmol/l butyrate and 5 mmol/l of propionate. However, when VCAM-1 expression was induced by TNF-α, preincubation with 2 and 4 mmol/l butyrate was shown to reduce this expression. In addition, TNF-induced adhesion of premonocytic U 937 to HUVECs was reduced by this treatment [41]. These findings are in line with those of Zapolska-Downar *et al.* [43,44•]: preincubation of HUVECs with propionate and butyrate (0.1, 1.0, and 10.0 mmol/l) for 24 h reduced VCAM-1 surface expression and mRNA levels in TNF-α and IL-1β-stimulated cells in a dose-dependent manner. Also, TNF-α-induced and IL-1β-induced adhesion of freshly isolated monocytes and lymphocytes to HUVECs was significantly reduced by treatment with both SCFAs (10 mmol/l, 24 h). The pretreatment with SCFA also reduced ICAM-1 surface expression and mRNA levels, though the effect was less pronounced than that on VCAM-1 [43,44•]. In contrary, no inhibitory effect on TNF-α-induced ICAM-1 expression was observed by Menzel *et al.* [41].

Taken together, these results indicate a clear inhibition of TNF-α and IL-1β-stimulated VCAM-1 expression by SCFA in HUVEC. In addition, butyrate could decrease the expression of the adhesion molecules ICAM-1 and lymphocyte function-associated antigen 3 (LFA-3) in monocytes [45]. This indicates that butyrate might prevent antigen-induced T-cell activation by preventing cell adhesion, which is essential for antigen-presenting cell (APC)-induced activation. Moreover, the same study demonstrated reduced expression of the co-stimulatory molecule B7-1 and upregulation of B7-2, indicating that butyrate might modulate T-cell activation by affecting co-stimulatory molecules [45].

By preventing chemotaxis and cell adhesion, SCFAs might prevent infiltration of immune cells in peripheral tissues and can have a protective effect against systemic inflammation. It would be interesting to see whether SCFAs can affect the migration of immune cells in

adipose tissue of obese people and whether SCFAs could prevent the infiltration of macrophages in arteries. SCFAs could by this means, in addition to their hypocholesteremic effect, contribute to the prevention of atherosclerosis [46].

Effects of short-chain fatty acids, especially butyrate, on the expression of cytokines in leukocytes

In the late 1980s and early 1990s, Eftimiadi *et al.* [47,48] already showed interaction of anaerobic bacteria derived SCFAs on the function of leukocytes. They reported impaired chemotaxis and reduced phagocytic activity by leukocytes. However, most studies on the immunomodulating effects of SCFAs were performed on epithelial cells in the context of inflammatory bowel disease. As immune cells are major contributors to these diseases, Säemann *et al.* [49] studied the effect of sodium butyrate on the cytokine expression of PBMC-derived monocytes. Butyrate inhibited *Staphylococcus aureus*-induced T_H1 cytokines IL-2 and IFN-γ and upregulated T_H2 cytokine IL-4 and anti-inflammatory IL-10 production. IL-12 and its receptor, important activators of IFN-γ production, were downregulated, resulting in inhibition of IFN-γ. Thus, butyrate induced a shift from a more proinflammatory T_H1-like profile to an anti-inflammatory profile. Another study showed similar results in PBMCs and the human acute monocytic leukemia cell line (THP-1) macrophage cell line, wherein butyrate inhibited LPS-induced inhibition of IκB degradation. In this way, butyrate prevented NF-κB translocation to the nucleus and hence it prevented transcription of the proinflammatory genes TNF-α, IL-1β, and IL-6. Similar results were recently reported by Park *et al.* [50]. Inducible nitric oxide synthase (iNOS), responsible for nitric oxide production in leukocytes, was also shown to be inhibited by butyrate in macrophages [39••,50–52].

These results indicate a clear anti-inflammatory effect of butyrate in leukocytes. Data of Cavaglieri *et al.* [53] suggest that this anti-inflammatory effect is mainly caused by butyrate, as butyrate reduced IFN-γ release, whereas propionate and acetate increased IFN-γ release. Peculiarly, propionate and acetate increased IL-10 expression, whereas butyrate had no effect. Incubation with a combination of butyrate and acetate or propionate still showed inhibition of IFN-γ release, whereas a combination of the three SCFAs led to an increase in IFN-γ, suggesting that the effects of different SCFAs are exerted via different mechanisms. A combination of the three SCFAs showed a slightly reduced proinflammatory profile compared to cultures without SCFAs [53]. Recently, Vinolo *et al.* [54•] showed butyrate-impaired neutrophil function, marked by decreased phagocytosis of *Candida albicans* and decreased killing activity via reduced production of reactive oxygen species. These parameters were not changed by propionate or acetate. Other studies, in contrast, showed anti-inflammatory effects of propionate and acetate [50,55,56,57•].

Säemann *et al.* [49] suggested that in peripheral lymphocytes and monocytes, butyrate prevents a proinflammatory and T_H1 profile and shifts it to a T_H2 profile, a phenomenon also reported for propionate [57•]. However, this is conflicting with other data, where, in a LPS-stimulated and phytohemagglutinin-stimulated whole-blood model, the butyrate-induced shift away from a proinflammatory and T_H1 profile did not result in a clear T_H2 profile [58]. Butyrate significantly reduced expression of LPS-induced IFN-γ, TNF-α, and IL-12 expression; however, IL-5 and IL-13, considered to be T_H2 cytokines, were also reduced. As cell viability was not affected by LPS or butyrate, apoptosis was not responsible for this overall cytokine downregulation as shown in a recent study of Bailón *et al.* [59••]. They showed downregulation of a large range of cytokines, both T_H1 and T_H2 by butyrate. This effect was dependent on the proliferative and differentiation status of the different types of cells (primary murine lymphocytes and monocytes and RAW 264.7 macrophages). In addition, the observed effects might also be concentration-dependent. An increase in IL-10 was reported for 0.5–1 mmol/l butyrate, though this effect disappeared at higher concentrations (4 mmol/l) [59••]. Säemann *et al.* [49] reported a dose-dependent increase in IL-10 upon incubation till 0.25 mmol/l; however, at higher concentrations (0.5–1 mmol/l), this effect disappeared. Prostaglandin E2 (PGE_2), a prostanoid that reduces LPS-induced TNF-α expression, was induced by butyrate at 1 and 2 mmol/l, but this effect also disappeared at higher concentrations of 5 and 10 mmol/l [56]. On the other hand, other studies show only effects of SCFA in lymphocytes at extremely high concentrations of 30 mmol/l. As physiological concentrations of butyrate and propionate beyond the colon are in the low micromolar range, experimental concentrations of 10 mmol/l or even 30 mmol/l are far from physiological and relevance of effects become questionable. As we will discuss later, SCFA can induce apoptosis in neutrophils and lymphocytes at concentrations much lower than 30 mmol/l. This implies that effects seen with high concentrations of butyrate and propionate might be due to toxicity, rather than a physiological relevant effect. Therefore, we suggest to carefully choose the concentration range of SCFA in the study of lymphocytes.

Mechanisms of butyrate-modulated cytokine expression

Several studies looked into the effect of stress and inflammatory signaling pathways as effector mechanisms of butyrate. Especially NF-κB was studied and was found

to be reduced in most studies [50,55,56,60]. Butyrate exerted this effect on NF-κB by preventing the proteasomal degradation of ubiquinated IκB; thus, NF-κB remained sequestered in the cytoplasm [55,60]. In addition to the effect of butyrate on NF-κB activation, several studies analyzed the effect of butyrate on the MAPK pathways. These mitogen-activated protein kinases (MAPKs) respond on extracellular stimuli like stress and cytokines and they regulate cellular processes as growth, proliferation, apoptosis, and inflammation via various cascades; major MAP kinases are ERK-1/2, JNK, and p38. Park *et al.* [50] demonstrated inhibition of ERK-1/2, whereas Diakos *et al.* [61] showed inhibition of JNK in mast cells, but could not show inhibition of NF-κB. In contrast, Bailón *et al.* [59••] did not see an effect of butyrate on NF-κB nor the MAP kinase ERK, JNK, or p38 in bone marrow-derived macrophages.

Butyrate is a known histone deacetylase (HDAC) inhibitor [62]. HDAC prevents gene transcription by keeping chromatin in a compact form, so inhibition by butyrate results in hyperacetylation. Via this mechanism butyrate can modulate gene expression and exerts an antiproliferative effect. It, therefore, attracted much attention for its potential role in fighting colon cancer [63]. It was also shown that the complex mechanism of acetylation and deacetylation can modulate NF-κB transcriptional activity via proteins in the NF-κB pathway as well as in modulating the accessibility of the NF-κB target genes [64]. IL-5, a typical T_H2 cytokine, is upregulated by butyrate-induced acetylation at its promoter site [65]. Butyrate and propionate repress LPS-induced TNF-α expression by upregulation of PGE_2 and cycloogygenase 2 (COX-2) via inhibition of HDAC in PBMCs and RAW 264.7 [40•,56,66]. On the other hand, butyrate strongly reduced COX-2 activity in the epithelial HT-29 colon cancer cell line, indicating that butyrate has a cell type specific effect [67].

Although the results are ambiguous, butyrate seems to have an anti-inflammatory effect, mediated by signaling pathways like NF-κB and inhibition of HDAC. The discrepancies in the results are most likely explained by the differences in cell types used and their proliferative and differentiation status.

The role of short-chain fatty acids in proliferation and apoptosis

Butyrate has a long history as HDAC inhibitor [68,69] and this function is thought to be involved in the effect of butyrate on proliferation and differentiation [70,71]. Butyrate can affect the rate of cell growth, DNA synthesis and it can induce growth arrest in the G1 phase of the cell cycle [70,72]; however, these effects differ per cell type. As butyrate affects proliferation, it has a more distinct impact on proliferating cells than on differentiated cells with a low rate of proliferation. Comalada *et al.* [73] showed that butyrate significantly inhibited proliferation and increased differentiation and apoptosis in the HT-29 carcinoma cell line, whereas normal, differentiated epithelial cells were not affected. Recently, this group elegantly showed that inhibition of proliferation and induction of apoptosis was dependent on the type of cell used, and more specifically its proliferation state [59••]. Low concentrations of butyrate were able to inhibit proliferation in all tested immune cells (primary splenic derived T cells, primary bone marrow-derived macrophages, and the murine RAW 264.7 macrophage cell line) and nondifferentiated HT-29 cells. As T-cell clonal expansion is required for activation, butyrate completely inhibited cytokine production, whereas in normal macrophages, which stop proliferating when activated, butyrate did not affect activation. Butyrate also induced apoptosis in proliferating cells, whereas nonproliferating macrophages and differentiated epithelial cells were hardly affected. These results indicate that the immune-modulatory effects of butyrate are dependent on the proliferation and differentiation status of the used cell type and that induction of apoptosis might be proliferation-dependent [59••]. Apoptosis was mediated by caspases; however, involvement of NF-κB and MAPK pathways was inconclusive. This is in agreement with Aoyama *et al.* [74••], who demonstrated participation of caspases, but ruled out the involvement of MAPKs as well as GPR41 and GPR43 in butyrate and propionate-induced apoptosis in neutrophils. In their study, acetate did not induce neutrophil apoptosis, in contrast to that in the study by Maslowski *et al.* [35••] who recently demonstrated GRP43-dependent apoptosis in neutrophils induced by acetate. These differences might be explained by the use of a $GPR43^{-/-}$ mice by Maslowski *et al.* [35••], whereas Aoyama *et al.* [74••] performed in-vitro studies on human neutrophils. As Aoyama *et al.* excluded GPR41 and GPR43 as possible mediators of SCFA-induced apoptosis, this might imply that there are other SCFA receptors present, which also could account for the differences in immune-modulatory and HDAC-inhibiting functions by SCFAs [53].

The apoptosis-inducing effect of SCFA also points to possible toxicity in in-vitro studies. Aoyama *et al.* [74••] show decreased cell viability of neutrophils after incubation with 4 and 10 mmol/l butyrate or propionate, but not with 0.4 mmol/l. Stehle *et al.* [75] demonstrated that 1 mmol/l butyrate and propionate could delay apoptosis, whereas 5 and 30 mmol/l accelerated apoptosis. As pointed out before, the effects of SCFA are highly dependent on the type of SCFA and its concentrations. Concentrations used in in-vitro studies highly deviate from in-vivo blood concentrations. Therefore, the choice of SCFA and concentration should be carefully evaluated when designing a study.

Conclusion

Overall, SCFAs, especially butyrate, seem to exert broad anti-inflammatory activities by affecting immune cell migration, adhesion, cytokine expression as well as affecting cellular processes such as proliferation, activation, and apoptosis. Recently, it became clear that another HDAC inhibitor may enhance numbers and function of T_{reg} cells, a population of T cells that suppress activity of the immune system [76,77]. As several studies showed increased butyrate-induced expression of IL-10, a modulator of T_{reg} cell function, it would be interesting to see whether butyrate has the same effect on T_{reg} cells and whether it can manipulate the balance between detrimental T_H1 and cytotoxic $CD8^+$ T cells versus T_{reg} cells; disturbance of this balance is more and more thought to be central in obesity-associated and systemic inflammation [27•,30•,78]. In addition, SCFA might prevent infiltration of immune cells from the bloodstream in peripheral tissues, for example, adipose tissue. Given that there are clues that obesity-associated inflammation might be antigen-mediated, and as butyrate was shown to induce T-cell anergy, SCFAs might be good candidates to evaluate in the fight against obesity-associated and systemic inflammation in general.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 760–761).

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