Intestinal dysbiosis and innate immune responses in axial spondyloarthritis

Francesco Ciccia, Angelo Ferrante, and Giovanni Triolo

Purpose of review
Inflammatory innate and adaptive immune cell responses to commensal bacteria underlie the pathogenesis of human chronic inflammatory diseases. Intestinal dysbiosis has been described in patients with spondyloarthritis (SpA) and seems to be correlated with histologic and immunologic alterations. Purpose of this review is to discuss the relationship occurring between intestinal dysbiosis and innate immune responses in patients with axial SpA.

Recent findings
Intestinal dysbiosis and differential activation of intestinal immune responses in patients with SpA have been demonstrated. Furthermore, innate cells that appear to be involved in the pathogenesis of SpA may control intestinal homeostasis through induction of apoptotic cell death and deletion of activated commensal bacteria-specific T cells.

Summary
Although the evidence shows that dysbiosis occurs in SpA, it is not clear the role of dysbiosis in regulating innate immune responses in SpA. Relationships between cause and effect remain to be answered.

Video abstract
http://links.lww.com/COR/A34.

Keywords
dysbiosis, gut inflammation, IL-17, IL-23, IL-9, innate lymphoid cells, spondyloarthritis

INTRODUCTION

Complex communities of microorganisms, termed 'microbiota', regulating host nutrient metabolism, immune cell homeostasis, and also protecting from pathogen infection, colonize the mammalian gastrointestinal tract [1▪▪]. The communities of bacteria, essentially located in the lower intestine, are separated from body tissues by the epithelial layer. The invasion of host tissue by resident bacteria may induce inflammation and sepsis. This is prevented by the intestinal immune system, including epithelial cells, that acts by complex interactions between the host and the microorganisms preserving this symbiotic relationship [2].

Intestinal dysbiosis has been demonstrated in the gut of patients with spondyloarthritis (SpA) [3▪,4▪,5,6▪▪] and it is accompanied by differential activation of Paneth cells (specialized cells in the epithelium of the small intestine) [7,8▪▪] that are an important source of antimicrobial peptides in the intestine [9] and by a dysregulation of proinflammatory cytokines [10▪▪,11] and of innate lymphoid cells [12▪▪]. This article will focus on recent studies implicating the role of intestinal bacteria in regulating immune responses in SpA patients.

Intestinal dysbiosis in patients with spondyloarthritis

Intestinal dysbiosis has been demonstrated in patients with SpA with different results in different subsets. In psoriatic arthritis (PsA), a lower relative abundance of multiple intestinal bacteria and, in particular, of beneficial taxa, such as Akkermansia, Ruminococcus, and Pseudobutyrivibrio has been demonstrated. These changes are associated with an increase in secreted IgA and decrease in receptor

Dipartimento Biomedico di Medicina Interna e Specialistica, Sezione di Reumatologia, Università degli Studi di Palermo, Palermo, Italy

Correspondence to Professor Giovanni Triolo, MD, Department of Internal Medicine, Division of Rheumatology, Piazza delle Cliniche 2, 90127 Palermo, Italy. Tel/fax: +390916552137;
e-mail: giovanni.triolo@unipa.it

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KEY POINTS

- Intestinal dysbiosis occurs in different subsets of SpA patients.
- Paneth cells are differentially activated in the gut of SpA patients.
- Activation and expansion of innate lymphoid cells characterizes the gut of ankylosing spondylitis.
- Activation and expansion of Th9 cells characterizes the gut of PsA patients.

activator of nuclear factor kappa-B ligand levels [3▪]. In enthesitis-related arthritis, a recent study [4▪] performed in children demonstrated less Faecalibacterium prausnitzii and Lachnospiraceae family, a statistically significant increase in bifidobacterium and an abundance of Akkermansia muciniphila. In ankylosing spondylitis, a decrease in the Bacteroides-Provotella and Clostridium leptum groups with an increase in Bifidobacterium has been observed [5]. Recently, Costello *et al.* by performing culture-independent microbial community profiling, demonstrated difference in the gut microbiome of terminal ileum biopsy specimens between ankylosing spondylitis patients and healthy controls. Ileal microbial communities of patients with ankylosing spondylitis differed significantly from healthy controls, being characterized by higher abundance of five bacteria families: *Lachnospiraceae, Veillonellaceae, Prevotellaceae, Porphyromonadaceae, and Bacteroidaceae* [6▪▪]. Altogether these findings indicate the presence of different associative bacterial agents in SpA. In this regard, further studies are required to specifically address the role of intestinal dysbiosis in the pathogenesis of SpA and the influence of host genetics in changing intestinal microbial composition.

Epithelial alterations in the gut of patients with spondyloarthritis

The intestinal epithelium forms a physical and biochemical barrier to commensal and pathogenic microorganisms conserving host–microbial interactions and tissue homeostasis [9]. This homeostasis depends on the capacity of intestinal epithelium in physically segregating commensal bacteria and integrating microbial signals. Alteration of intestinal permeability is associated with the passage of viable resident bacteria from the gastrointestinal tract to normally sterile tissues such as the mesenteric lymph nodes, and the translocation of inert particles and other macromolecules, such as lipopolysaccharide endotoxin, across the intestinal mucosal barrier [9]. Alteration of intestinal permeability has been demonstrated in patients with ankylosing spondylitis and their first relatives degree [13] and preliminary data seem to indicate downregulation of the tight junction proteins occludin, claudin 2 and 4, and zonula occludens 1 in ankylosing spondylitis ileum [14]. These alterations of tight junctions are presumably associated with increased intestinal levels of zonulin [14]. Zonulin, the only physiological modulator of intercellular tight junctions described, is involved in trafficking of macromolecules and, therefore, in the balance between tolerance and immune responses [15]. Zonulin expression seems to be modulated by intestinal bacteria and may represent a host response against bacterial colonization of the small intestine [16]. According to the increased permeability, preliminary evidences suggest that high serum levels of lipopolysaccharide and i-fatty acid binding protein are present in ankylosing spondylitis, significantly associated with the level of tissue inflammation [14]. It is not clear, however, whether or not these alterations are related to specific microorganisms.

Role of Paneth cells in modulating intestinal immune responses

It has been demonstrated that certain secreted antibacterial proteins produced by epithelial cells can shape the composition of intestinal microbial communities. For example, α-defensins small (2–3 kD) antibacterial peptides secreted by Paneth cells have been proved to modify overall community composition in mice [17]. Paneth cells are highly specialized epithelial cells of the small intestine. They are located just below the intestinal stem cells in the crypts of Lieberkühn and display large eosinophilic refractile granules that occupy most of their cytoplasm. These granules contain several antimicrobial compounds and other compounds that are known to be important in immunity, in the maintenance of crypt sterility and regulating the balance with colonizing microbiota and enteric pathogens [18]. Paneth cells have been also demonstrated to participate in the regulation of several pathways of innate and adaptive immunity, including that of IL-23/IL-17 axis [18]. In the presence of bacteria or bacterial antigens, Paneth cells secrete these peptides into the lumen of the crypts of Lieberkühn, thereby contributing to maintenance of the gastrointestinal barrier. Paneth cells seem also to be important in the regulation of intestinal inflammation as seen in ileal Crohn's disease that seems to be a specific disorder of Paneth cells [19]. Homozygosity for the highly prevalent *ATG16L1* risk allele causes Paneth cell dysfunction and deletion of the unfolded protein

response (UPR) transcription factor X-box binding protein-1 in intestinal epithelial cells, results in endoplasmic reticulum (ER) stress, Paneth cells impairment, and spontaneous enteritis [19]. Paneth cells are activated in the gut of patients with SpA and produce different cytokines in different subsets of SpA. In ankylosing spondylitis, Paneth cells are activated, produce high levels of antimicrobial peptides, such as α-defensin 5, phospholipase A2, and lysozime [7] and are an important source of IL-23 [10▪▪], a key cytokine involved in the pathogenesis of disease, and of IL-32 a cytokine produced in response to bacterial products (Fig. 1) [19,20]. In patients with PsA, a hyperplasia of activated Paneth cells is present, as suggested by the increased levels of antimicrobial peptides such as α-defensin 5 present in the inflamed ileum [8▪▪]. Differently from ankylosing spondylitis, in PsA gut Paneth cells are characterized by the expression of IL-9 and not of IL-23 (Fig. 2) [8▪▪]. IL-9 is a cytokine initially purified and characterized as a T and mast cell growth factor that impairs intestinal barrier function and prevents mucosal wound healing *in vivo* [21▪▪]. Interestingly, in PsA gut Paneth cells also express the specific IL-9 receptor and the stimulation of isolated epithelial cells with IL-9 upregulates the expression of the antimicrobial peptides α-defensin 5 and of cytokines, such as IL-23 and IL-9, suggesting the possibility of a functional autocrine loop involving IL-9/IL-9R (Fig. 2) [8▪▪]. According to the studies discussed below, Paneth cells seem to play a fundamental role in regulating innate intestinal immune responses, presumably in response to altered microbiota in SpA patients.

Autophagy and unfolded protein response in spondyloarthritis gut

In the context of Paneth cells, the innate pathway of autophagy senses microbial products to activate

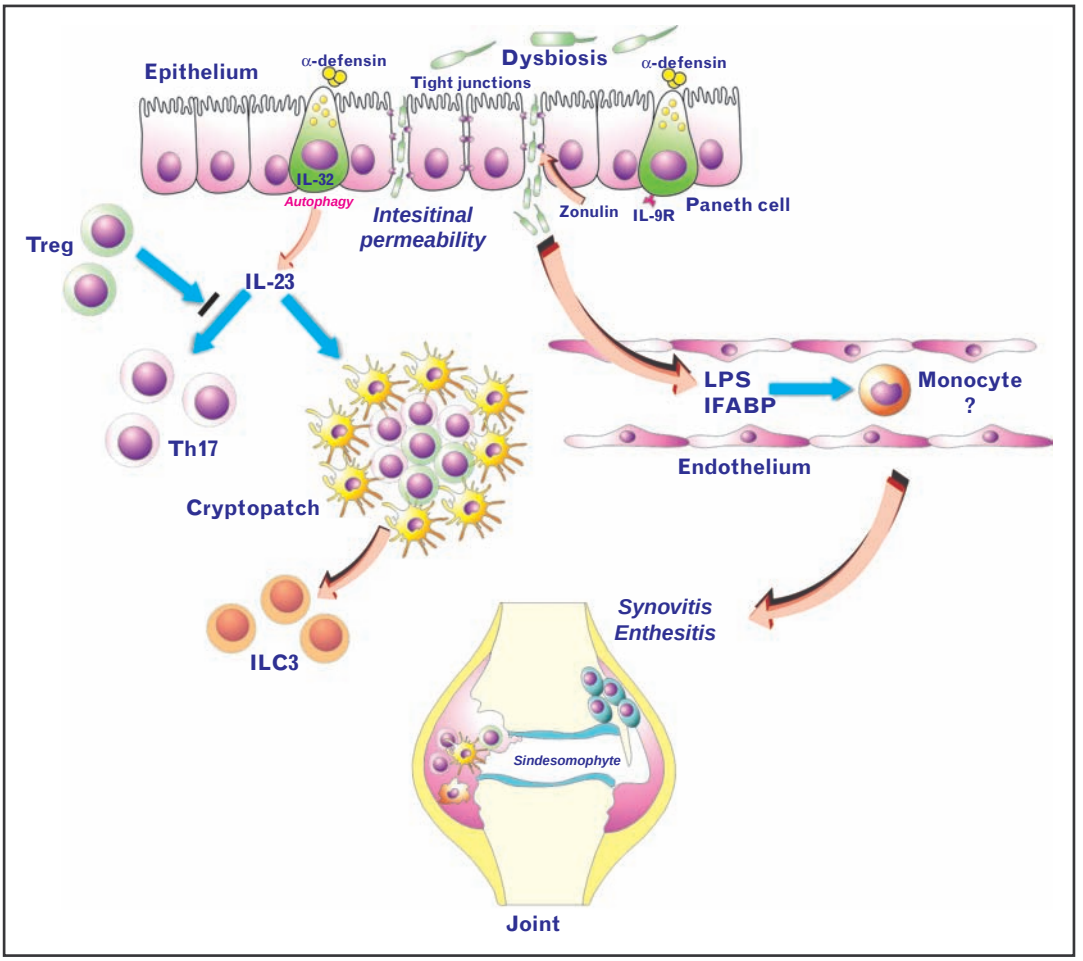

FIGURE 1. Intestinal dysbiosis and immune responses in ankylosing spondylitis. Dysbiosis in ankylosing spondylitis induces alterations of intestinal permeability and over-expression of IL-23 that may lead to the generation of Group 3 innate lymphoid cells (ILC3s). ILC3 may recirculate in the sites of inflammation of ankylosing spondylitis.

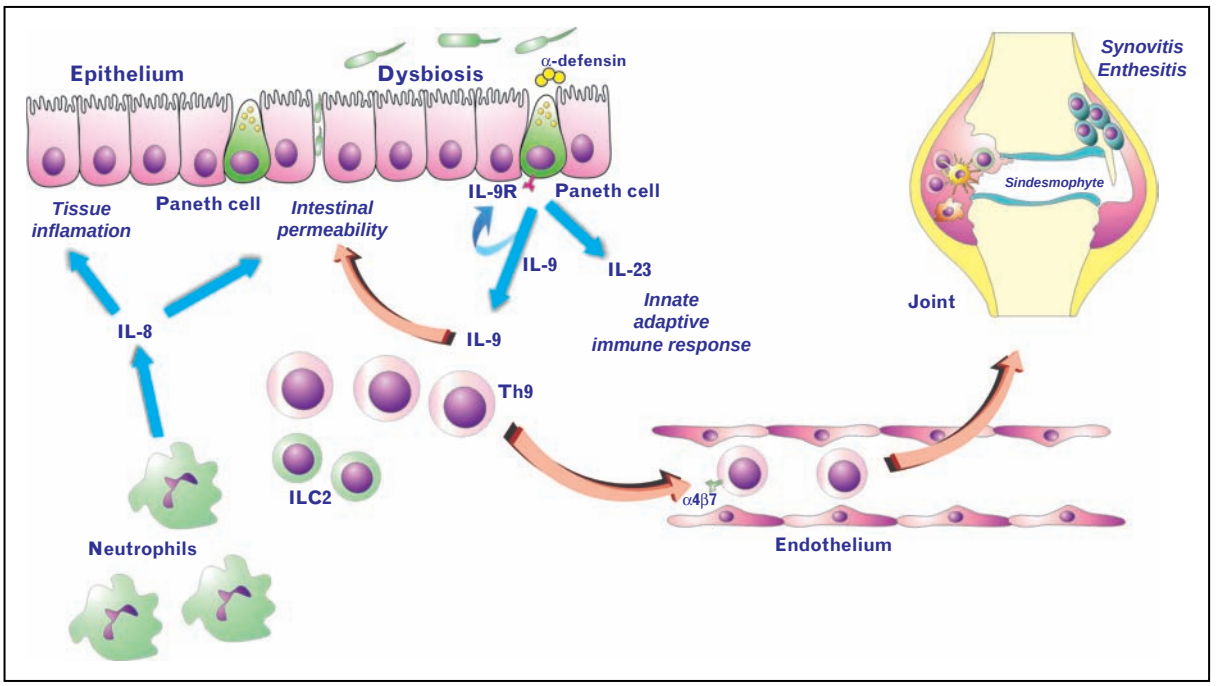

FIGURE 2. Intestinal dysbiosis and immune responses in psoriatic arthritis. Dysbiosis in psoriatic arthritis is associated with the over-expression of IL-9 and Th9 cell polarization. Gut-derived Th9 cells, may recirculate in the sites of inflammation of psoriatic arthritis.

inflammatory processes and, concomitantly, interact with cellular stress responses such as the UPR [22]. Impairment in either UPR or autophagy function in Paneth cells results in each other's compensatory engagement, with development of severe spontaneous Crohn's disease-like transmural ileitis if both mechanisms are compromised [19]. Unresolved ER stress, in response to HLA-B27 misfolding, has been observed in the gut of HLA-B27 transgenic rats but not in HLA-B27 ankylosing spondylitis patients, in which, however, some studies [23–25] indicate the occurrence of ER stress in peripheral blood mononuclear cells/monocytes. In ankylosing spondylitis in HLA-B27 misfolding seems to occur as demonstrated by the intracellular colocalization of SYVN1 and free heavy chains (a marker of active free heavy chains misfolding) [26▪▪]. Despite the presence of HLA-B27 misfolding, there was not, however, a significant overexpression of UPR genes. Differently from UPR, upregulation of the genes involved in the autophagy pathway is present in the gut of ankylosing spondylitis patients, mainly characterized by an increased expression of *LC3II*, *ATG5*, and *ATG12* [27]. In particular, LC3II a marker of ongoing autophagy, is expressed among infiltrating mononuclear cells and Paneth cells, colocalizing with ATG5. Further, autophagy but not UPR was required to modulate the expression of IL-23 in isolated LPMCs of ankylosing spondylitis patients with chronic gut inflammation [26▪▪]. Autophagy activation seems to be a tissue-specific process in ankylosing spondylitis, since that activation of autophagy has been not confirmed in the peripheral blood and synovial tissues in ankylosing spondylitis patients [27].

Innate immune responses in the gut of spondyloarthritis

Innate immune activation occurs in the gut of SpA patients as demonstrated by the aberrant expression of cytokines and expansion of specific subset of innate cells. Nucleotide-binding oligomerization domain-containing protein 2 (Nod2) has been extensively characterized as a bacterial sensor that induces an antimicrobial and inflammatory gene expression program [28]. In humans, activation of monocytes via NOD2 (by its ligand muramyl dipeptide) induced differentiation of dendritic cells, which is dependent on an IL-32-dependent mechanism [20]. IL-32 is a recently described cytokine that has been thought to be a proinflammatory player because of its capacity to induce the maturation and activation of dendritic cells and the production of T helper (Th)1 and Th17-polarizing cytokines via a phospholipase C/c-Jun N-terminal kinase/nuclear factor-kappaB-dependent pathway [29]. IL-32 is constitutively expressed in epithelial cells of colon mucosa and stimulated by IL-1β, IFN-γ, and TNF-α and synergizes with nucleotide

oligomerization domain (NOD)1 and NOD2 ligands for IL-1β and IL-6 production, thus acting as a proinflammatory cytokine [30]. IL-32 is strongly upregulated in the inflamed ileum of ankylosing spondylitis patients at both mRNA and protein levels. In particular, IL-32 is intensely expressed in the context of Paneth cells, among endothelial cells of lamina propria vessels, in the inflammatory infiltrates (where Crohn's disease 68+ cells were the major cellular source) and in the germinal centers of gut-associated lymphoid tissues in both ankylosing spondylitis and Crohn's disease patients. Interestingly, in ankylosing spondylitis IL-32 expression is correlated with IL-10 levels and the in-vitro exposure of intestinal epithelial cell lines to IL-32 determined an increased IL-10 production [11]. These findings suggest that in ankylosing spondylitis, IL-32 participates in epithelial tissue protective responses in which the production of IL-10 and antimicrobial peptides could be the main actors (Fig. 1).

To prevent the translocation of commensals and/or pathogenic microorganisms across the epithelial barrier, the intestinal tract is equipped with a highly complex intrinsic immune system that includes tissue-specific organized lymphoid structures, such as Peyer's patches, cryptopatches, and isolated lymphoid follicles [31,32]. Isolated lymphoid follicles formation is induced postnatally by commensal microbiota and requires cryptopatches. Cryptopatches are lymphoid structures containing lymphoid tissue inducer (LTi) cells, a distinct population of group 3 innate lymphoid cells [33▪▪,34]. In response to microbiota signals, intestinal epithelial cells produce IL-7 that signals via the IL-7 receptor on LTi cells to induce the expression of lymphotoxin α1β2 (LT-α1β2) [33▪▪,34,35] that acts in turn on resident lymphoid tissue organizer cells, upregulating the expression of chemokines (e.g. CXCL13, CCL19, and CCL21) and adhesion molecules (e.g. VCAM1 and ICAM1) required for the recruitment and retention of lymphocytes into the cryptopatches [35]. In the inflamed gut of ankylosing spondylitis patients IL-7 is overexpressed, especially in the context of Paneth cells, and aggregates of LTi cells (CD3 c-kit$^+$/IL-7R$^+$) resembling cryptopatches are present in close proximity of the bottom of intestinal crypts (Fig. 1) [12▪▪]. Cryptopatches seem to be absent in normal ileal samples suggesting that, in physiological conditions, they may exist only during a small window of time just after birth or, alternatively, that they need bacterial stimulation to develop. The important role of intestinal epithelial cells in modulating the behavior of LTi cells is also suggested by the ability of isolated epithelial cells from the gut of ankylosing spondylitis to induce the differentiation of type III ILCs [12▪▪]. ILCs are different populations of cells with lymphoid characteristics but without rearrangement of antigen receptors, mainly distinguished into three groups: IFN-γ-producing ILC1 cells, T helper type 2 cytokines producing ILC2 cells, and IL-17 and IL-22 producing ILC3 cells that are associated with the gastrointestinal mucosa [34]. It has been recently demonstrated that ILC3 express major histocompatibility complex class II (MHCII), similarly to thymic epithelial cells, and that MHCII(+) ILC3s directly induce cell death of activated commensal bacteria-specific T cells. MHCII on colonic ILC3s has been demonstrated to be reduced in pediatric inflammatory bowel disease patients suggesting that this selection pathway for commensal bacteria-specific CD4(+) T cells is dysregulated in human inflammatory bowel disease [36▪▪]. In ankylosing spondylitis patients, ILC3 are expanded in the gut and characterized by the high expression of IL-17 and IL-22 and of the transcription factor *T-bet* (Fig. 1) [12▪▪]. Absence of RAR-related orphan receptor C in ankylosing spondylitis intestinal ILC3 could be related to a specific stage of differentiation of these cells as it has been demonstrated that ILC3s may follow a differentiation program in which they first upregulate *T-bet*, then acquire natural cytotoxic receptors and then downregulate *RORγt*, indicating phenotypic and functional plasticity [34]. It is not possible to confirm that different subsets of ILC3s producing different cytokines may act promoting different innate immune responses. It seems reasonable, however, that ILC3s producing IL-22 might be involved in tissue protective responses such as the induction of goblet cells hyperplasia and increased mucins production, observed in ankylosing spondylitis gut. Conversely, IL-17 and IL-17/IL-22-producing cells might be involved in eliciting proinflammatory activities. ILC3s have been also demonstrated to be expanded in the peripheral blood, synovial fluids, and bone marrow of ankylosing spondylitis patients and to express the intestinal homing integrin α4β7 (Fig. 1) [12▪▪]. In addition, MAdCAM1, the α4β7 ligand, was found to be highly represented in the high endothelial venules of the gut and in the inflamed bone marrow (BM) of ankylosing spondylitis, suggesting that a recirculation of ILC3 between the gut and the BM occurs. In light of this evidence, modulation of ILC3s might be considered as a therapeutic strategy in ankylosing spondylitis. In this regard, antitumor necrosis factor therapy resulted in ankylosing spondylitis in the reduction of ILC3 at both systemic and gut level and in a strong reduced expression of MAdCAM1 in ileal samples [12▪▪], indicating that therapeutic modulation of ILC3 may be beneficial in ankylosing spondylitis.

Adaptive immune responses in the gut of spondyloarthritis patients

Different immune polarization is present in ankylosing spondylitis and PsA gut. In ankylosing spondylitis patients, despite IL-23 overexpression, a clear Th17 and Th1 polarization is lacking [10▪▪]. Conversely, subclinical gut inflammation of PsA patients is characterized by clear Th17 and Th22 polarized immune responses [8▪▪]. Unlike ankylosing spondylitis, a strong and significant upregulation of IL-9 immunologically was observed in PsA gut, mainly expressed by Th9 cells and high endothelial venules [8▪▪]. Th9 subset of helper T cells has been demonstrated to serve an important role in driving intestinal inflammation in ulcerative colitis [37▪] and the significant expansion we observed in PsA patients may indicate a role for these cells also in PsA gut inflammation (Fig. 2). Th9 are expanded also in the peripheral blood and in synovial tissues of PsA and express $\alpha4\beta7+$ indicating the intestinal origin of these cells (Fig. 2) [8▪▪]. These findings may suggest a different immune polarization in the gut of ankylosing spondylitis vs. PsA patients. Whether these alterations are driven by the different microbiota remains, however, to be elucidated.

CONCLUSION

Intestinal dysbiosis is present in SpA patients and is associated with alterations of intestinal permeability and extensive activation of innate and adaptive immunity. Modulation of microbiota and/or of innate immune responses may warrant future therapeutic strategies in SpA.

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Conflicts of interest

There are no conflicts of interest.

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