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2 **cGAS-STING DNA-sensing in Inflammatory Bowel Diseases**

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11

12 **Keywords:** Inflammatory bowel diseases; intestinal mucosal damage; cell death; ectopic DNA
13 release; DNA sensing; cGAS-STING pathway;

14

15

16 **Abstract**

17 Inflammatory Bowel Diseases (IBD) are chronic, incurable pathologies with unknown
18 causes affecting millions of people. Pediatric-onset IBD, starting before age of 18, are
19 increasing, with more aggressive and extensive features than adult-onset IBD. These
20 differences remain largely unexplained. Intestinal mucosal damage, cell death, DNA
21 release from nuclear, mitochondrial or microbiota sources, and DNA sensing activating
22 cGAS-STING pathway may contribute to the disease evolution. Increased colonic cGAS
23 and STING starts to be reported in experimental and human IBD. However, limited
24 knowledge of the mechanisms involved hinders the development of new therapeutic
25 options. Here we discuss recent advances and unresolved questions regarding DNA
26 release, DNA sensors activation, and the role and therapeutic potential of the cGAS-
27 STING pathway in inflammatory colitis.

28

29

30

1 **Current knowledge of inflammatory bowel diseases**

2 **Inflammatory Bowel Diseases** (IBD, see Glossary), including **Ulcerative colitis** (UC)
3 and **Crohn's disease** (CD), are chronic incurable pathologies of unknown aetiology
4 [1,2] affecting millions of people all over the world [3,4]. Intestinal microbiota alterations,
5 nutrition and immune factors trigger gut inflammation in genetic predisposed adults and
6 children [5]. Common symptoms are chronic diarrhoea, abdominal pain, malnutrition
7 complicated sometimes by fistulisation, stenosis and perforation [6] associated to
8 important psychological impact [7]. Intestinal lesions are generated by infiltrates of
9 activated immune cells in gut wall which are responsible for imbalanced proinflammatory
10 cytokines secretion [2,8]. Anti-inflammatory, corticosteroid, nutritional, immune-
11 suppressive and biologic therapies are currently used to achieve partial remission [1,9],
12 while refractory forms do not respond to any treatment [10,11], and the risk of
13 inflammation-induced colonic cancer is increased [12]. Research and exploration of new
14 biological pathways are critical to better understand IBD physiopathology, possible
15 cause(s), identify new targets and potential drugs and curative treatment.

16

17 **Damage of the intestinal mucosa barrier integrity and DNA release in IBD**

18 Mucosal damage in IBD is a hallmark feature of both Crohn's disease and ulcerative
19 colitis. It results of chronic inflammation of the gastrointestinal (GI) tract and has various
20 causes, manifestations, and consequences. IBD are characterized by an inappropriate
21 immune response to intestinal microbiota. This immune response leads to the release
22 of pro-inflammatory **cytokines** (e.g., TNF α , IL-6, IL-12) that damage the mucosal lining.
23 Pro-inflammatory cytokines such as TNF α can induce apoptosis in epithelial cells,
24 contributing to tissue damage [13-15]. Cell death and the release of extracellular DNA
25 play significant roles in the pathogenesis of IBD [16]. Elevated levels of extracellular
26 DNA are found in the serum and tissues of IBD patients and mice [17,18]. The
27 extracellular DNA can originate from apoptotic bodies, necrotic cells, and **neutrophil**
28 **extracellular traps (NETs)** [19,20]. **Necroptosis** is a programmed form of necrosis
29 mediated by receptor-interacting protein kinases (RIPK) and mixed lineage kinase
30 domain-like protein (MLKL) [21]. This pathway can be triggered by TNF α under caspase-
31 inhibited conditions and contributes to inflammation in IBD [22-24]. **Pyroptosis** is a form
32 of programmed cell death associated with inflammation, characterized by the activation

1 of caspase-1 and gasdermin D [25]. It results in the release of pro-inflammatory
2 cytokines and **DAMPs**, amplifying the inflammatory response in IBD [26].

3 **DNA sensing and cGAS – STING pathway**

4 Since two decades the pro-inflammatory functions of extranuclear DNA has been
5 recognized. Cytosolic self-DNA fragments, released from nucleus and/or mitochondria
6 due to cell damage or genomic instability, as well as foreign microbial DNA, interact with
7 molecular sensors and trigger inflammatory processes. Beyond endosomal **TLR9**, the
8 main cytosolic DNA sensors are the interferon-gamma inducible protein IFI16 and the
9 cyclic GMP–AMP synthase cGAS even if several others may have similar function such
10 as the DExD/H-box helicases DHX9 and DHX36, DDX41 and DDX60, and AIM2 [27,28].
11 Since the discovery of STING [29] and cGAS [30], the exploration of cGAS-STING
12 pathway raised interest in innate immunity signalling and new molecular aspects are
13 being unravelled (**Figure 1, Key Figure; see Text Box 1**) [31-33]. Functional in
14 prokaryotes, invertebrates, vertebrates and humans, the first evolutionary role of cGAS-
15 STING pathway was anti-viral protection. Further studies showed its implications in
16 autoimmunity such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis,
17 neurodegeneration, senescence and cancer [32,34].

18

19 **cGAS and STING in experimental colitis**

20 ***Is cGAS – STING pathway activated in experimental colitis?***

21 The most widespread model of murine colonic inflammation is acute DSS-induced colitis
22 [35,36], usually evaluated with the **Disease Activity Index** (DAI) and body weight
23 change [37], for studying both CD and UC. Several reports associated DSS-colitis with
24 cGAS and STING activation and induction Type I IFN response transcript and protein
25 [31,38-42].

26 Excessive cytosolic DNA triggers cGAS-STING pathway. Deficiency of DEAH-box
27 helicase 9 (DHX9), a protein that unwinds RNA or DNA duplexes and RNA-DNA hybrids,
28 aggravates chemical colitis/inflammation by increasing R-loops and genomic instability,
29 promoting cGAS-STING activation [41]. A decrease of colonic DNase I activity was
30 reported in murine DSS-colitis [43].

31 *Data on colonic inflammation in cGAS-deficient murine models are controversial:*
32 attenuated inflammatory responses in DSS colitis [42], versus STING-mediated Type I

1 IFN colonic cell signaling [40]. cGAS blockade by intraperitoneal injection of the cGAS
2 inhibitor Ru.521 significantly attenuated DSS-induced colitis in terms of weight loss, DAI
3 score, shortening in colon length, and histopathological findings [44].
4 The role of STING in murine models of colitis seems ambiguous.
5 *On the one hand, triggering STING promotes colitis.* Agonist-induced STING
6 activation by DMXAA worsened DSS-colitis [45]. *In vitro*, natural STING ligands such as
7 bacterial c-di-AMP or mammalian 2'3'-cGAMP, as well as DMXAA promote the
8 polarization of M2 macrophages to the inflammatory M1 phenotype in human THP-1-
9 derived macrophages [45]. STING signalling drives pro-inflammatory, MyD88-
10 dependent gene induction in murine bone marrow-derived macrophages (BMDMs) and
11 dendritic cells (BMDCs) [46]. Moreover, constitutive activation of STING (N153s)
12 resulted in chronic *spontaneous* colitis at 1.5-6 months of age in mice [47]. Conversely,
13 DSS-induced colitis was attenuated in STING deficient mice [39,48]. STING signalling
14 may also play a significant role in the development of colitis in the absence of IL-10,
15 possibly by interacting with microbes to generate pro-inflammatory cytokines [46].
16 Recently, Zhang et al. demonstrated *in vitro* in THP1 and MEF cell lines **aryl**
17 **hydrocarbon receptor (AHR)**-dependent nuclear translocation of STING resulting in
18 *anti-inflammatory effect* in presence of microbiota-derived tryptophan metabolites [48].
19 AHR ligand-induced *CYP1A1* (cytochrome P450 family 1 subfamily A member 1) and
20 *IL22* expression was STING-dependent. *In vivo*, oral tryptophan metabolites had anti-
21 inflammatory effect and corrected intestinal **dysbiosis** in DSS-colitis murine model [48].
22 Additional data are awaited to confirm this nuclear pathway.
23 *On the other hand, STING may attenuate colitis,* as complete STING deficiency [49] or
24 cell-specific STING loss in splenic CD4⁺T cells [50] increased colitis severity in DSS-
25 colitis model. The suggested mechanism by which STING exhibits anti-inflammatory
26 action involved the stimulation of IL-10 production [46,50]. In murine BMDMs and
27 BMDCs the expression of IL-10 is modulated by STING signalling [46]. STING induces
28 IL-10 in splenic T-cells via glucose and glutamine metabolism, and enhances
29 mitochondrial oxidation in Th1 cells by activating mitochondrial STAT3 [50]. A competing,
30 non-canonical nuclear translocation of STING was demonstrated after exposure to aryl
31 hydrocarbon receptor (AHR) ligands that limited the activation of the cGAS-STING1-
32 IRF3 pathway by sequestering STING away from the ERGIC *in vitro* [48]. Microbiota-
33 derived tryptophan metabolites AHR ligands triggered AHR-dependent nuclear
34 translocation of STING, yielding STING-dependent expression of AHR target genes

1 *CYP1A1* (cytochrome P450 family 1 subfamily A member 1) and *IL22* [48]. In vivo, oral
2 tryptophan metabolites had anti-inflammatory effects and corrected intestinal dysbiosis
3 in DSS-colitis murine model [48]. Therefore, the colitogenic activity of cGAS-STING
4 pathway is context-dependent.

5
6 Interference with cGAS-STING pathway in experimental colitis indicated a decrease of
7 intestinal epithelial cell (IEC) inflammatory status (**Figure 2**). *IGF2* administration
8 resulted in decrease of colonic STING in DSS-context associated to upregulation of
9 barrier integrity protein markers Occludin and ZO-1 and improving of colonic stemness
10 [31]. Atrial Natriuretic Peptide (*ANP*) inhibited STING pathway and repaired gut barrier
11 damage in DSS colitis model [38]. *In vitro* in HT-29 IECs, ANP promoted cGAS/NPR-A
12 complex formation, inhibited ER stress-induced autophagy, STING pathway activation
13 and repaired gut barrier damage. Brefeldin-a (*BFA*) was associated with improvement
14 of DSS-induced colitis, possibly by inhibiting STING pathway and NLRP inflammasome,
15 but causality is not proven [42]. *In vitro* BFA decreased transcript and protein levels of
16 the cGAS-STING pathway and NLRP3 inflammasome in human LPS-induced SW480
17 IECs [42]. Low-dose ganciclovir (*GCV*) ameliorated DSS-induced colitis, possibly by
18 inhibiting cGAS-STING signaling [39] but no molecular interaction is shown. In addition,
19 blockade of EVs by *GW4869*, an inhibitor of EVs release, alleviated inflammation in
20 murine colitis by inhibiting the activation of the STING pathway [18]. Thus, there is
21 promising experimental data of interference on cGAS-STING pathways alleviating
22 parameters of colitis.

23
24 ***cGAS – STING pathway activation contributes to colonic epithelial cell integrity***
25 ***and gut barrier dysfunction***

26 Little is known about cGAS in the colonic epithelium. In a DSS-colitis model, cGAS
27 deficiency increases colonic epithelial cell death (TUNEL assay) without reducing
28 MUC5AC or goblet cells [40,42]. *In vitro*, GFP-tagged cGAS interacts with Beclin-1,
29 upregulating autophagy and increasing the LC3II/I ratio in HCT-116 cells. Rapamycin
30 enhances autophagy in both cGAS-transfected and control HCT-116 cells [40].
31 However, more data are available on STING. In a constitutive STING-activated N153s
32 mouse model, loss of colonic goblet cells is observed.

1 The population of colonic epithelial cells expressing EpCAM/CD326 was similar in 4.5-
2 month-old N153s mice and wild type controls, indicating no link between STING
3 activation and colonic epithelial cell death. Increased antimicrobial peptide secretion
4 (DEFA1, REG3G, DEFA25) and decreased colonic mucus proteins (MUC2, Tff3) were
5 observed [47]. This model showed altered gut barrier function, with elevated intestinal
6 permeability, reduced tight junction proteins (ZO-2, Claudin-2), and increased colonic
7 fibrosis. Mice with constitutive STING activation had more neutrophils and
8 CD3⁺TCRβ⁺CD4⁺ cells, fewer T-cells and CD3⁺TCRβ⁺CD8⁺ cells, but stable levels of
9 CD3⁺TCRβ⁺CD4⁻CD8⁻, macrophages, monocytes, eosinophils, and basophils in colonic
10 epithelium compared to controls [47].

11 In human colonic HT-29 IECs, IL-22 promoted DNA damage and γH2AX upregulation
12 via ER stress, leading to cytosolic dsDNA release and STING pathway activation in the
13 absence of ATG16L1, indicating defective autophagy [51]. STING deficiency resulted in
14 reduced IFNβ, crypt height, goblet cells, mucus layer (MUC1 & MUC2), fecal secretory
15 IgA, colonic IL-1β protein, and Amphiregulin, but intestinal permeability and IL-22bp
16 expression remained unchanged [49].

17
18 ***Colonic Lamina Propria Cells rely on cGAS – STING pathway in experimental***
19 ***colitis***

20 Lamina propria cells (LPC) play a key role in colonic inflammation, with most being
21 immune cells from blood and lymph. Their excessive presence indicates colitis. The
22 cGAS-STING pathway in LPC myeloid cells, stimulated by DNA fragments, induces
23 IFNβ production. Without STING, LP Innate Lymphoid Cells (ILC) type 1 and ILC3
24 increase, while ILC2, IL-4, and IL-5 decrease [49]. The frequency and function of LP T-
25 regulatory (Treg) cells, IL-10, and TGFβ also drop. Yang et al. found reduced colonic LP
26 IL-10⁺CD4⁺ T cells in STING-deficient mice, with unaffected IL-10 production. The
27 frequencies of Th1, Th2, and Th17 cells remain unchanged. Additionally, depleting colon
28 phagocytes with clodronate liposomes eliminated colitis in N153s [47]. The absence of
29 TCRδ⁺ T cells did not protect from colitis, whereas the absence of TCRβ⁺ T cells
30 reduced colonic inflammation.

31 cGAS plays a crucial role in intestinal LP cell-induced inflammation, particularly in
32 myeloid cells during DSS-colitis. Gasdermin D (GSDMD), a key pyroptosis executioner,
33 negatively regulated cGAS-mediated inflammation [44]. GSDMD deficiency

1 exacerbated DSS-colitis by increasing cGAS-dependent inflammation in LP colon
2 macrophages. Inhibition of cGAS in GSDMD KO mice reduced colitis, highlighting the
3 protective role of GSDMD in controlling cGAS-mediated inflammation [44]. Additionally,
4 a non-canonical AHR-dependent (anti-inflammatory) STING pathway, involving colonic
5 ILC3 and Th17 invasion, was recently associated with DSS colitis [48].

6

7 ***Intestinal microbiota shaped by cGAS – STING pathway in experimental colitis***

8 cGAS deficiency altered gut microbiota composition in a DSS murine model but not at
9 steady state. DSS-colitis mice without cGAS showed significant dysbiosis with
10 decreased alpha-diversity, distinct beta-diversity, and notable differences at genera and
11 species levels [42]. Antibiotic-induced dysbiosis activated cGAS and triggered a type I
12 IFN response in the DSS-colitis model [42]. cGAS played a functional role in shaping
13 gut microbiota and was needed to regulate microbiota expansion in colitis. cGAS
14 deficiency increased colonic inflammation severity without affecting microbiome beta-
15 diversity. STING-deficient and wild-type mice had the same microbiota without
16 experimental colitis [52]. In vitro, intestinal commensal microbiota activated STING and
17 induced a type I IFN response in MEFs culture [49].

18

19 The absence of STING in experimental colitis was linked to dysbiosis, increased
20 *Turicibacter* and *Odoribacter*, a pro-inflammatory microbiota with more Proteobacteria
21 (especially *Desulfovibrio*), reduced Actinobacteria (*Bifidobacterium*), Firmicutes
22 (*Allobaculum*), lower Shannon alpha-diversity, and colonic carcinogenesis [46,49,50].
23 IL-10 has been suggested, but not confirmed, as a factor in the suppression of colon
24 inflammation [49,50]. Gong et al found that STING-deficient mice were protected from
25 DSS-induced dysbiosis [39]. An alternative explanation involves AHR-mediated
26 microbial regulation affecting *Lactobacillus* and *Helicobacter*, leading to a healthy, non-
27 inflammatory gut microbiota via a non-canonical STING pathway [48]. This highlights
28 molecular crosstalk between STING and microbiota metabolites such as FICZ, ITE, and
29 L-KYN, resulting in AHR target gene transcription, particularly CYP1A1, which promotes
30 homeostasis by metabolizing polyunsaturated fatty acids and xenobiotics.

31

32 In constitutively STING-activated N153s mice, the bacterial profile of the microbiota was
33 pro-inflammatory. Broad-spectrum antibiotic treatment abolished colitis and made

1 colonic STING undetectable. Targeted antibiotic treatment to deplete *Helicobacter*
2 *typhlonius*, a bacteria elevated in this model, also abolished colitis and reduced STING
3 expression [47]. Fecal microbiota transplantation (FMT) from N153s to WT DSS 1%
4 mice exacerbated colitis and increased colonic STING protein levels, an effect absent
5 in STING-deficient mice [47]. STING activation and stabilization in this model was
6 proposed to occur via post-transcriptional K63-linked ubiquitination in colonic LP
7 myeloid CD11b⁺ cells following intestinal dysbiosis, as confirmed in vitro on BMDMs
8 cultured with c-di-GMP [47].

9

10 **Cross-talk between autophagy, ER stress and cGAS – STING pathway in colitis**

11 Autophagy is a major protective mechanism in IBD and experimental colitis [31,53]. In
12 experimental colitis canonical cGAS-STING pathway induced IFN I production,
13 autophagy activation [48] and endoplasmic reticulum stress [38]. cGAS deficiency was
14 associated with down-regulation of ATG16L1 (marker of autophagy) [42] and decreased
15 Beclin-1-mediated autophagy during intestinal inflammation [40]. Interaction of cGAS
16 and Beclin-1 upregulated autophagy in murine IECs but also in vitro in human colonic
17 IECs (HCT-116) [40].

18 Restoration of autophagy via rapamycin treatment dampened DSS-induced colitis
19 severity in cGAS KO mice [40]. In human colonic IECs HCT-116 rapamycin promoted
20 autophagy independently of cGAS [40]. Defective autophagy by ATG16L1 absence in
21 IEC aggravated DSS colitis [51] which is not affected by systemic IL-22 treatment (but
22 promoted ileitis) or anti-IFNAR Antibodies (which alleviated histological *ileitis* and
23 reduces *ileal* cell death by activation of cGAS – STING pathway) [51]. In human colonic
24 IECs (Caco-2 cells) IL-22 promoted STING pathway (increase of pTBK1 protein)
25 activation in absence ATG16L1 [51]. Atrial Natriuretic Peptide Inhibiting STING pathway
26 was associated to blockade of ER Stress-Induced Autophagy and restoration of gut
27 barrier damage in DSS-colitis mice [38].

28

29 **Cell death by apoptosis or pyroptosis regulated by cGAS – STING pathway in** 30 **colitis**

31 cGAS deficiency was associated with increased colonic epithelial cell death by TUNEL
32 staining after DSS exposure [40], with increased level of cleaved caspase-3 [40] and

1 decrease of STAT3 [42], suggesting upregulation of apoptosis. Loss of cGAS was
2 correlated with upregulated expression of Caspase 1 indicating pyroptosis induction
3 [42]. Gasdermin-D (GSDMD), major pyroptosis player, was highly activated in DSS-
4 colitis even though GSDMD had no impact on the production of anti-microbial peptides
5 (AMPs) such as Reg3b, Reg3g, Defn5 and Lysozyme1, and mucus MUC2. As GSDMD
6 deficiency drove severe DSS-colitis in a microbiota-independent manner, protective role
7 of GSDMD in DSS-colitis was dependent on its controlling cGAS-mediated inflammation
8 especially in colonic LP macrophages, and to lesser extent in colonic LP Neutrophils, by
9 promoting their death [44].

10 Constitutive STING N153s activation was associated with increase of *splenic* CD4⁺ cell
11 death by apoptosis attested by increased levels of cleaved PARP. This phenomenon
12 was *not* observed on colonic epithelial cells and colonic LP CD4⁺ cells [47]. STING
13 activation was linked to epithelial cell death in small intestine [51] but no data are
14 available about colon.

15 In human healthy epithelial colonic organoids type I IFN treatment increased STING,
16 IFI16, ZBP1, inflammasome gene expression while combined treatment with IFN- β and
17 TNF- α induced Caspase-independent cell death [54].

18

19 **Nutrition effect on cGAS – STING pathway in colitis**

20 Specific nutrition effect on cGAS-STING pathway in DSS-colitis murine model. Oxidized
21 triglyceride monomers bearing epoxy moiety, generated during deep-frying oil,
22 aggravated intestinal epithelial cell pyroptosis through Caspase-1/NLRP3/GSDMD [55].
23 Epoxy triglycerides also activate cGAS-STING signaling pathway, yielding
24 proinflammatory cytokines and enhanced intestinal permeability by disintegration of tight
25 junction proteins occludin and zonula occludens-1 (ZO-1), promoting intestinal
26 metabolic homeostasis imbalance in DSS-induced colitis [55]. Whether mislocalized
27 cGAS from the damaged inner cell membrane [56] is involved in enhancement of this
28 process, is still unknown.

29

30 **Increased ectopic, cell-free DNA in experimental colitis**

31 Although cGAS-STING pathway is intracellular, its activity is positively associated with
32 the presence and amount of ectopic, cell-free DNA (cfDNA). The latter depends on cell

1 damage or death leading to expulsion of DNA out of nucleus and mitochondria, active
2 secretion of DNA fragments by EVs, extracellular DNase activity, and probably NETosis.
3 Total plasmatic cfDNA increased in murine colitis [18,20,43,57,58]. Plasmatic cfDNA
4 from nuclear and mitochondrial origins was upregulated in murine DSS-colitis models
5 [20], and was essentially localized in exosomal vesicles [18]. Moreover, abnormally
6 elevated dsDNA levels in EVs from plasma and colon positively correlated with score
7 severity DAI. Blockade of EVs by the inhibitor of EVs release GW4869 alleviated
8 inflammation in murine DSS-induced colitis by inhibiting the activation of the STING
9 pathway [18]. Both the murine DSS model and active human CD colitis showed a
10 substantial rise in exosomal dsDNA levels, including mitochondrial DNA and nuclear
11 DNA (nDNA). In vitro EVs containing dsDNA from damaged colonic epithelial cells in
12 LPS-treated murine CT26 cells were internalized by murine macrophages to induce type
13 I IFN production, while STING deficient macrophages were not activated. Human EVs
14 from active CD patients activated STING pathway in murine macrophages in a dsDNA
15 dependent manner, while no STING pathway activation was observed by EVs from
16 inactive CD and controls [18].

17
18 Extracellular DNA fragments length 50 to 250 bp of mitochondrial, nuclear, bacterial, or
19 viral origin produced same effects on DSS-treated and healthy mice [20]. DSS-exposed
20 mice preconditioned by cfDNA injections demonstrated improved mucosal and
21 splenocyte autophagy and significantly attenuated colonic inflammation [59]. Whether
22 this effect was mediated by TLR9, cGAS-STING or other pathways remains to be
23 elucidated.

24 NETotic cells, another potential source of cfDNA, were also significantly increased in the
25 plasma of DSS-exposed mice [20,60] while NETosis markers (NE, PAD4, MPO) were
26 upregulated in the colonic tissue [20,58,60,61]. Inhibition of NETosis by Cl-amidine or
27 streptonigrin intraperitoneal injection partially ameliorated 2%DSS-colitis observed by
28 significant decrease of endoscopic score [20], but no change of DAI and cfDNA [20].
29 Authors reported a decrease of colonic DNase activity [43], but no difference was
30 observed about extracellular plasmatic DNase activity in DSS-colitis mice versus
31 controls [20,43]. To our knowledge, no direct link between NETosis and cGAS-STING
32 pathway in colitis has been demonstrated yet. Nevertheless, DNase I intravenous
33 injection in mice with experimental colitis significantly alleviated colonic inflammation
34 clearly suggesting the inflammatory function of NETs DNA [61].

1

2 **Elevated cGAS and STING pathway in clinical IBD**

3 ***Differences in pediatric vs adult IBD***

4 The epidemiology and phenotype in children and adults suffering from IBD is different,
5 and studying the involvement of the cGAS-STING pathway may be particularly relevant
6 not only in adults but also in children IBD. Pediatric-onset IBD (PO-IBD), starting before
7 age of 18, represent up to 20-25 % of all IBD nowadays. Unlike adult-onset IBD, PO-
8 IBD show a continuous increased incidence in industrialized countries [4,62] with more
9 aggressive extensive features [63]. For instance, PO-IBD exhibit pancolitis at diagnosis
10 in 90% of PO-UC in children versus 37% at adulthood [64-66], global inflammatory
11 activity of 40% in PO-IBD versus 30% in AO-IBD [67,68], and family history of IBD in
12 30% PO-IBD versus 15% AO-IBD [63,69]. These phenotypical differences remain
13 largely unexplained. Therefore, characterizing the potential involvement DNA sensing
14 and cGAS-STING pathway in colonic IBD pathophysiology could be a precious piece of
15 the puzzle of the adult and/or intestinal inflammation and a new therapeutic target.

16

17 ***Elevated cGAS – STING in Ulcerative Colitis***

18 Eleven studies exploring role of cGAS-STING pathway in UC comprise 369 UC patients
19 and 194 healthy controls (**Table 1**) [17,38-40,42,47,50,51,54,57,58].

20 Human colonic cGAS is upregulated in UC [39,40,42] and its colonic level in active UC
21 is higher than in remission UC patients [38,54]. Similarly, elevation of colonic STING is
22 reported in UC [38-40,47,54], with higher pSTING in active and inactive UC as
23 compared to controls [38]. ISG were upregulated in colonic tissue of active UC patients
24 [51]. A meta-analysis [39] reported increase of colonic IFNAR2, proinflammatory IL-1 β
25 but no change in IFNAR1 expression, IFN α 2, IFN β 2 in UCs. Colonic anti-inflammatory
26 IL-10 level was elevated [39,50] due to STING stimulation of CD4⁺T cells likely limiting
27 the pathogenicity of Th1 cells to maintain intestinal homeostasis [50]. Colonic STING
28 expression, increased in CD4⁺T cells, correlated with IL-10 expression in UC [50].
29 Colonic upregulation of IL-22 expression, previously reported in IECs cell cultures as
30 responsible for nuclear DNA damage and STING pathway promotion [51], was
31 confirmed in active UC patients [51]. DNA sensors ZBP1 and IFI16 were also
32 overexpressed in colonic biopsies of active UCs [54]. Plasma cfDNA of nuclear and

1 mitochondrial origin was increased in UCs [17,57,58] and positively correlated to clinical,
2 NETs marker neutrophil elastase, and histological activity in UCs of children and adults
3 [58].

4 5 ***Elevated colonic cGAS – STING in Crohn's Disease***

6 Six clinical studies exploring role of cGAS-STING pathway in CD comprised 209 human
7 patients and 145 healthy controls (**Table 2**) [17,18,39,42,51,57].

8 In CDs, colonic STING was upregulated and cGAS-STING pathway was enriched
9 [18,39]. Macrophages CD68⁺pSTING and CD68⁺pIRF3 were significantly predominant
10 in active CD versus inactive CDs colonic infiltrate [18]. As in UCs, in CDs was reported
11 increase of ISGs expression (especially in active forms) [51], colonic IFNAR2,
12 proinflammatory IL-1 β , but no change in IFNAR1 expression, IFN α 2, IFN β 2. IL-1 β [39]
13 and IL-22 elevation [51] were associated with increased colonic secretion of IL-10 [39]
14 likely to counterbalance inflammation. Plasma cfDNA of nuclear and mitochondrial origin
15 was upregulated in active versus remission CDs [18] and in remission CDs versus
16 healthy controls [17,57]. Moreover, EVs containing dsDNA activated STING pathway in
17 macrophages to promote the development of CD [18]. Colonic type II IFN γ and its
18 receptor IFNGR1 were also upregulated [39].

19

20 ***Shared DNA sensing features in UCs and CDs data***

21 Plasma DNase I activity decreased in male IBD in remission [17]. Fecal **mtDNA** was
22 increased in IBD [57]. Colonic IL-22 expression correlated with colonic ISG score and
23 with IBD activity [51]. IBD Remission after anti-TNF correlated with decrease of colonic
24 IL22, TNF, necroptosis marker MLKL mRNA and ISG score [51]. IL-22–ISG axis might
25 be involved in therapy response to anti-TNF treatment in IBD patients [51]. Decrease
26 of colonic DHX9 protein in UC and CD patients led to increase of cytosolic DNA and
27 cGAS-STING activation, while transcripts were similar to healthy controls [41].
28 Hyperactivation of caspase-7, a major pro-apoptotic factor degrading DHX9, was
29 highlighted in vitro but remains to be proven in humans [41]. Another potential
30 therapeutic target, the enzyme amino adipate aminotransferase (AADAT), delivering
31 beneficial tryptophan metabolites and probably activating AHR-STING pathway, was
32 lower in IBD patients versus controls, and first animal experiments are encouraging [70].

33

1 **Concluding Remarks**

2 In conclusion, although cGAS-STING pathway is involved in murine and human IBD
3 colitis, its precise role in colonic inflammation requires further investigation (**Figure 3**).

4 As intestinal inflammation in human IBD induces cell damage, DNA release, DNA
5 sensors mobilization, activation of cGAS – STING pathway may contribute to promote
6 immune cell influx and M1 polarization of lamina propria macrophages. Further, gut
7 epithelium barrier rupture favors contact between microbiota microorganisms and
8 metabolites with immune cells in intestinal wall. Thus, we propose that the consecutive
9 auto-amplification loop of inflammation mediated by cGAS-STING pathway might
10 contribute to IBD chronic evolution.

11 The interactions between microbiota composition, microbial metabolites, and the cGAS-
12 STING pathway in the colon are not fully understood. Additionally, the translation of
13 findings from murine models to human conditions presents significant challenges.

14 The paucity of human data on cGAS-STING pathway in IBD underscores the need for
15 increased collaboration between clinicians and immunologists (see **Outstanding**
16 **questions**). Furthermore, the influence of human nutrition, especially in genetically
17 predisposed individuals, warrants special attention as a potential trigger for colitis.
18 Advances in modern biological technics, especially OMICs approaches such as single
19 cell sequencing focused on human gut barrier, hold promise for elucidating the role of
20 the cGAS-STING pathway in IBD pathophysiology. Finally, the development and testing
21 of potential drugs targeting cGAS and/or STING are critical next steps in this research
22 area (see **Clinician's corner**).

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11 **Resources**

12 i <https://clinicaltrials.gov/study/NCT00639821> ;

13 ii <https://clinicaltrials.gov/study/NCT00783718?tab=history&a=10>

14 iii NCT00790933 <https://www.clinicaltrialsregister.eu/ctr-search/trial/2008-002784-14/SE>

15 iv <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16879>

16 v <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE37283>

17 vi <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52746>

18 vii <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59071>

19 viii <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ebcdycqsxfcdrap&acc=GSE73661>

20 ix <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75214>

21 x <https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA642084>

22 xi <http://ibdmdb.org>

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Text Box 1: Recent advances in DNA sensing and cGAS – STING pathway

Origins of cytosolic dsDNA fragments are foreign extracellular (bacterial, viral, fungal) and/or self-DNA in situation of cell stress or death [32,34]. Under homeostatic conditions small amounts of cytoplasmic DNA are eliminated by nucleases - DNase1 and DNase1L3 accounting for most DNase activity in blood [71,72]. Cell damage from e.g. infection, inflammation or genomic instability, yields nuclear and/or **mitochondrial DNA** fragments in the cytosol.

Mechanistically, in mammalian cells, cytosolic enzyme cGAS binds to dsDNA which leads to the production of the second messenger 2'3' cyclic GMP-AMP (cGAMP) which may be transferred to neighbouring cells by gap junction [73]. cGAMP senses the Endoplasmic Reticulum (ER) protein STING resulting in STING dimerization and coatamer protein complex II (COPII)-mediated translocation to the Golgi where it interacts with **TANK-binding kinase (TBK1)** and activates its autophosphorylation. Downstream pathway has two directions: On the one hand, TBK1 phosphorylates interferon regulatory factor 3 (IRF3) leading to its dimerization which enters the nucleus, binds DNA and promotes transcription of type I interferons (IFN) and IFN stimulated genes (ISG). On the other hand, phosphorylated TBK1 activates the **NFκB** pathway inducing the production of proinflammatory cytokines [31-33]. Control mechanisms of activation include cellular compartmentalization [74], cytosolic dsDNA length (human cGAS being activated by dsDNA fragments exceeding 40 bp), excess of cytosolic dsDNA, post-transcriptional modifications of pathway components [31,33] and deficiency of cytosolic DNase activity [75]. STING localisation on inner nuclear membrane was reported [76]. cGAS is also present into the nucleus where it is inactivated by chromatin [34,77-83] and may be degraded [84], as on the inner cell membrane [56], but its exact function on those locations is unknown. DNA-damaged chromatin containing micronuclei were reported to inhibit cGAS-STING pathway in cytoplasm [85] but this need confirmation.

1 **Clinician's Corner**

- 2 • Inflammatory Bowel Diseases (IBD), including Ulcerative colitis (UC) and Crohn's
3 disease (CD), are chronic, incurable pathologies of unknown aetiology. Symptoms
4 include chronic diarrhoea, abdominal pain, malnutrition, fistulisation, stenosis and
5 perforation, with psychological impact. Intestinal lesions are due to infiltrates of activated
6 immune cells in gut wall with secretion of proinflammatory cytokines. Despite increasing
7 number of biotherapies and immunomodulators, control of intestinal inflammation
8 remains incomplete and transient. Refractory forms do not respond to any treatment,
9 increasing the risk of inflammation-induced colonic cancer.
- 10 • Intestinal mucosal damage, cell death, DNA release from nuclear, mitochondrial or
11 microbiota sources are hallmarks of IBD. Excess of ectopic DNA, notably due to
12 genomic instability, has been shown to promote colonic inflammation *in vitro* and *in vivo*.
13 One of cytosolic sensors of ectopic DNA, the cyclic GMP-AMP synthase (cGAS),
14 triggers stimulator of interferon genes (STING) leading to NFκB pathway activation and
15 type I interferon pro-inflammatory responses.
- 16 • The few available clinical studies report increased colonic cGAS and STING in human
17 UC and CDs, together with cell-free DNA of nuclear and mitochondrial origin, positively
18 correlating to clinical and histological activity in children and adults UCs.
- 19 • IBD epidemiology and phenotype in children and adults is different. Pediatric-onset IBD
20 (PO-IBD), starting before age of 18, are increasing (>20 % of all IBD) with higher and
21 more aggressive extensive features including pancolitis and global inflammatory activity.
22 These differences remain largely unexplained, and characterizing the involvement DNA
23 sensing and cGAS-STING pathway in colonic IBD pathophysiology may help improve
24 understanding.
- 25 • The influence of human nutrition, especially in genetically predisposed individuals,
26 warrants special attention as a potential trigger for colitis. Some intestinal microbiota
27 metabolites, especially originated from tryptophane degradation, are able to inhibit
28 STING-induced inflammation and restore homeostasis. Exploring cGAS-STING
29 pathway, its interactions with essential cellular processes such as autophagy and cell
30 death, gut microbiota and its metabolites, could shed a new light on the pathophysiology
31 of adult and pediatric-onset IBD. Moreover, targeting cGAS-STING pathway to redirect

- 1 it towards homeostatic state opens a new field of pharmacological interventions to
- 2 alleviate colitis symptoms in IBD.

1 Glossary

- 2 • **Aryl hydrocarbon receptor (AHR):** Cytoplasmic receptor complexed to chaperone
3 Heat Shock Protein HSP90 under steady-state conditions, AhR is translocated to the
4 nucleus upon binding to xenobiotic chemicals from dietary, microbial, and metabolic
5 origin such as dioxins, flavonoids, or kynurenine pathway metabolites, to act as a
6 transcription factor stimulating transcription of target genes such as xenobiotic-
7 metabolizing enzyme cytochrome P450.
- 8 • **cGAS:** This nucleotidyltransferase catalyzes the formation of cyclic dinucleotide
9 c[G(2',5')pA(3',5')p], 2'3'cGAMP upon DNA recognition, irrespective of the DNA
10 sequence.
- 11 • **Crohn's disease (CD):** Chronic incurable inflammation of gastro-intestinal tract,
12 possibly affecting from mouth to anus, sometimes accompanied by extra-digestive
13 symptoms.
- 14 • **Cytokines:** Cytokines are a group of signaling molecules produced by cells that control
15 the immune system and diseases.
- 16 • **DAMP:** Endogenous molecules released in response to cellular stress or cell damage
17 (such as uric acid, ATP, nucleic acids) triggering cell activation *via* inflammasomes or
18 other sensors.
- 19 • **Disease Activity Index (DAI):** Score used to evaluate the chemical colitis in
20 experimental murine models. DAI index is calculated as total score (body weight
21 decrease + stool consistency + rectal bleeding) divided by 3.
- 22 • **Dysbiosis:** Imbalance in intestinal microbiota bacterial composition or metabolic
23 activities.
- 24 • **Inflammasomes:** Members of the nucleotide-binding domain and leucine-rich repeat
25 (LRR)-containing (NLR) family and of the pyrin and HIN domain (PYHIN) family NLRP1,
26 NLRP3, NLRC4, Pyrin and absent in melanoma 2 (AIM2) form inflammasome
27 multiprotein complexes. Inflammasomes activate caspase-1 leading to the maturation
28 of IL-1 β and IL-18 and the induction of pyroptosis programmed cell death. NLRP6,
29 NLRP7, NLRP12 and IFI16 might also form inflammasomes.
- 30 • **Inflammatory Bowel Diseases (IBD):** Crohn's disease and Ulcerative colitis
- 31 • **Ulcerative colitis (UC):** Chronic incurable inflammation of colon.

- 1 • **Mitochondrial DNA (mtDNA):** Mitochondrial genome; small, unmethylated circular
2 double-stranded DNA molecule present in multiple copies. Due to its lack of protective
3 histones and limited repair capacity, mtDNA is more susceptible to ROS induced
4 damage than nuclear DNA, and considered a biomarker of oxidative stress.
- 5 • **Neutrophil extracellular traps (NETs):** release of DNA, histone and granular contents
6 into the extracellular space, after a unique form neutrophil cell death.
- 7 • **Necroptosis:** A regulated, pro-inflammatory, caspase-independent form of necrotic cell
8 death.
- 9 • **Pyroptosis:** Pro-inflammatory cell death resulting from caspase-1 activity leading to
10 membrane pores and proinflammatory cytokine processing.
- 11 • **NF- κ B:** Nuclear factor kappa-light-chain-enhancer of activated B cells is a protein
12 complex that controls the transcription of DNA, cytokine production, and cell survival.
- 13 • **STING:** Encoded by the *TMEM173* gene, this endoplasmic reticulum associated protein
14 recognizes cyclic dinucleotides such as cGAMP and induces type I IFN expression.
- 15 • **TANK-binding kinase (TBK1):** Serine/threonine kinase, member of IKK-kinase family,
16 is phosphorylated downstream of STING activation inducing type I IFNs and plays a role
17 in autophagy and mitophagy.
- 18 • **TLR9:** This is an endosomal receptor that detects viral and bacterial CpG DNA and self-
19 DNA.

1 **Figure Legends:**

2

3 **Figure 1: Recent advances in cGAS-STING pathway-mediated modulation of**
4 **intestinal inflammation.**

5 cGAS is activated by excessive cytosolic dsDNA. Extracellular vesicles containing
6 microbiota- or dead cell-derived dsDNA facilitate entry into cytosol. Genomic instability due
7 to stalled R-loop formation by decrease of DHx9 helicase associated to mitochondrial
8 damage induces intracellular dsDNA release. DNA sensing by cGAS results in production
9 of second messenger cGAMP and activation of STING pathway with phosphorylation of
10 STING, TBK1 and IRF3 leading to type I IFN, ISG transcription and NFkB activation leading
11 to inflammation. Proapoptotic caspase-7 degrades DHx9, increases genomic instability,
12 contributing to cGAS-STING mediated inflammation. Moreover, bacterial CDNs such as c-
13 di-AMP promote inflammation by activating STING directly. On the other hand, a
14 competitive, non-canonical pathway is driven by tryptophan degradation by amino adipate
15 aminotransferase (AADAT) producing AHR ligands which promote STING translocation to
16 the nucleus. The STING-AHR-PML transcriptional complex triggers production of AHR
17 targets such as cytochrome p450 CYP1A1 and IL-22, contributing to cellular homeostasis.
18 This figure was created using BioRender (<https://biorender.com/>).

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21 **Figure 2: DNA release and cGAS-STING pathway activation leading to M1**
22 **polarization in colonic inflammation.**

23 Colonic lamina propria macrophages engulf EVs containing dsDNA activating pro-
24 inflammatory cGAS-STING and leading to M1 macrophage polarization, colonic lesions,
25 caspase-1 and Gasdermin D mediated cell death and increase dsDNA release, with
26 possible upregulation of epithelial NETs. Those phenomena may be counterbalanced by
27 AHR ligands-induced competitive non canonical STING-AHR pathway, or modulated by
28 insuline growth factor 2 (IGF2), atrial natriuretic peptide (ANP), ganciclovir (GCV), Brefeldin
29 A (BFA) and T-regulatory lymphocytes. This figure was created using BioRender
30 (<https://biorender.com/>).

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1 **Figure 3: cGAS-STING pathway at the crossroad of homeostasis versus colonic**
2 **inflammation.** cGAS-STING pathway has a context-dependent activity. DsDNA release
3 increases cytosolic DNA pool while degradation by DNAses contributes to homeostasis.
4 Increased genomic instability and reduced DHx9, as bacterial CDNs, are important factors
5 activating cGAS-STING mediated inflammation. AHR ligands divert STING towards
6 STING-AHR complex, favoring anti-inflammatory activity. Increased colonic secretion of IL-
7 10 in IBD counteracts inflammation. Equilibrium of those elements contributes to colonic
8 homeostasis. This figure was created using BioRender (<https://biorender.com/>).

Table 1: Ulcerative Colitis Clinical Data including DNA release, cGAS/STING or Type I IFN responses

Ulcerative Colitis population sample (n, age, M/F)	Aim	Active UC (n, age, M/F)	Remission UC (n, age, M/F)	Healthy controls (n, age, M/F)	Study design	Biological sample	Main findings	Reference
7	Elucidate the role of intrinsic STING signalling in regulating Th1 cell function to maintain intestinal homeostasis	NA	NA	12	Observational study (case-control)	Colonic biopsies	Upregulation of STING and IL-10 mRNA in UC vs H	[50]
26	Explore the expression and function of ANP in UC, and its potential regulatory role in the STING pathway	15 Age 42 [20-62] M/F 9/6	9 Age 44 [23-58] M/F 5/4	45 Age 36 [23-61] M/F 25/20	Observational study (case-control)	Colonic biopsies	Upregulation of cGAS mRNA and protein in aUC vs rUC. Upregulation of p-STING, p-TBK1, p-IRF3 in aUC vs rUC, rUC vs H. No change of STING, TBK1, IRF3 mRNA in aUC, rUC and H.	[38]
64	Investigate the involvement of inflammasome, cell death and type I IFN signaling in UC by analysing expression of DNA sensor	33 Age 44±13.9 M/F 17/16	31 Age 44±13.9 M/F 15/16	31 Age 54±13.1 M/F 15/16	Observational study (case-control)	Colonic biopsies	Upregulation of cGAS mRNA in aUC vs rUC, aUC vs H. Upregulation of STING, TBK1, IRF3 mRNA in aUC vs H.	[54]
4 Age 47 [35-69] M/F 2/2 245 (metanalysis of 5 microarray studies)	Test the hypothesis that ganciclovir may have therapeutic effects on experimental colitis through acting on cGAS-STING signaling	NA 245 (metanalysis)	NA	4 Age 60 [57-78] M/F 2/2 41 (metanalysis)	Metanalysis of 5 microarray studies	Colonic biopsies	Upregulation of STING protein in 4 UC vs 4 H. Upregulation of cGAS, STING, IFNAR2, IL-1β and IL-10 mRNA in UC vs H. No change IFNAR1, IFNα-2 and IFNβ-2 mRNA levels in UC vs H. Anti-TNF Infiximab downregulated STING, not cGAS mRNA.	[39]: metanalysis of 5 microarray studies: [86-90]
8	Understand the role that cGAS plays during intestinal inflammation	8 Age 5-21	0	8 Age 5-21	Observational study (case-control)	Colonic resections	Upregulation of cGAS mRNA and protein in aUC vs H.	[40]
4	Understand functional role of STING in constitutively active mouse model of STING (N153s) to better understand human UC pathophysiology	4 Age NA M/F NA	0	3 Age NA M/F NA	Observational study (case-control)	Colonic biopsies	Upregulation of STING protein in aUC vs H.	[47]
25 Age 30 [6-76] M/F 10/15	Construct a gene-microbiota interaction-based framework to discover IBD biomarkers and therapeutics	NA	NA	22 Age 30 [6-69] M/F 12/10	Observational study (case-control)	Colonic biopsies	Upregulation of cGAS mRNA in UC vs H. cGAS as potential marker for IBD, especially UC.	[42]

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28 Age 40 [21–71] M/F 14/14	Analyze circulating cfDNA and deoxyribonuclease (DNase) activity in IBD patients in clinical remission	0	28 Age 40 [21–71] M/F 14/14	29 Age 27 [18–46] M/F 12/17	Prospective controlled	Plasma	Upregulation of nc-cfDNA and mt-cfDNA in rUC vs H. (no good markers for clinical remission, but no evidence of mucosal healing) Decrease of plasma DNase activity in Males vs Females in rUC.	[17]
13	Investigate whether IL-22 could beneficially modulate cellular function and epithelial gut homeostasis in situations of defective autophagy or ER stress.	6	7	0	Observational study (case-control)	Colonic biopsies	Upregulation of 'ISG score', IL-22, TNF, MLKL mRNA in aUC vs rUC. IL-22 mRNA positively correlated with colonic ISG score and IBD activity. IL-22 – ISG axis might be involved in therapy response by anti-TNF treatment in aUC.	[51]
67 Age 36 [28–51] M/F 44/23	Investigate whether mtDNA is released during active disease, serving as a key pro-inflammatory factor in IBD	NA	NA	40 Age 33 [27–46] M/F 23/17	Prospective controlled	Plasma Stools Colonic Colonic resections	Upregulation of plasma mt-cfDNA and mt-peptides, fecal mt-cfDNA and colonic LP TLR-9 protein in UC vs H.	[57]
123 Age 36 [3–75] (20 UC <15 y)	Explore whether plasma cf-DNA reflects inflammatory state of IBD	NA	NA	0	Prospective cohort	plasma	Good correlation between cfDNA increase and severe inflammation: clinic status, blood CRP, Neutrophils and NE, and histological severity.	[58]

Footnote: aUC: active ulcerative colitis; rUC: UC in remission; H: Healthy controls; cfDNA: circulating free DNA; LP: Lamina Propria; NE: Neutrophil Elastase. All cited studies received approval of the respective Institutional Ethical Boards, and are completed. Gong *et al.*, 2022: meta-analysis of CD colonic samples from NCT00639821ⁱ; NCT00783718ⁱⁱ; NCT00790933ⁱⁱⁱ; the microarray data are available at the GEO accession number GSE16879^v; GSE37283^v; GSE52746^{vi}; GSE59071^{vii}; GSE73661^{viii}; GSE75214^x. Schmucl-Galia *et al.*, 2021: The accession number for the whole-genome shotgun (WGS) DNA sequencing and 16s rDNA profiling is NCBI BioProject, ID: PRJNA642084^x. Wang 2021: Publicly available RNA-seq and microbiome data on human IBD are part of the Integrative Human Microbiome Project (iHMP), IBD Multi'omics database IBDMDB^{xi}.

Table 2: Crohn's Disease Clinical Data including DNA release, cGAS/STING or Type I IFN responses

Crohn's Disease population sample (n, age, M/F)	Aim	Active CD (n, age, M/F)	Remission CD (n, age, M/F)	Healthy controls (n, age, M/F)	Study design	Biological sample	Main findings	Reference
45 (Metanalysis of 4 studies)	Test the hypothesis that ganciclovir may have therapeutic effects on experimental colitis through acting on cGAS-STING signaling	45 Age NA M/F NA	NA	45 Age NA M/F NA	Metanalysis of 5 microarray studies:	Colonic biopsies	Upregulation of cGAS, STING, IFNAR2, IL-1 β , IL-10 mRNA in aCD vs H. No change IFNAR1, IFN α -2 and INF β -2 mRNA levels in aCD vs H. Anti-TNF(Infliximab) downregulated cGAS mRNA but not STING mRNA.	[39]: metanalysis of 5 microarray studies: [86-90]
43 Age 26 [8 – 76] M/F 22/21	Construct a gene-microbiota interaction-based framework to discover IBD biomarkers and therapeutics	NA	NA	22 Age 30 [6 – 69] M/F 12/10	Observational study (case-control)	Colonic biopsies	cGAS and STING mRNA trend to increase in CD vs H.	[42]
10 Age 45 [34.3-52] M/F 4/6	Investigate whether intestinal extracellular vesicles (EV) transport pathogenic mtDNA and nDNA to trigger innate immune responses through cGAS-STING pathway in CD	5	5	9 Age 47 [32.5-54] M/F 6/3	Observational study (case-control)	Plasma Colonic biopsies	Plasma mt-dsDNA and nc-dsDNA significantly increased in aCD. Plasma EV-dsDNA (mt- and nc-DNA) positively correlated with disease activity. Increased EVs secretion, STING & NFkB pathways activation, and colonic CD68 ⁺ pSTING ⁺ and CD68 ⁺ pIRF ⁺ Macrophages in aCD.	[83]
72 Age 41 [22–69] M/F 44/28	Analyze circulating cfDNA and deoxyribonuclease (DNase) activity in IBD patients in clinical remission	0	72 Age 41 [22–69] M/F 44/28	29 Age 27 [18–46] M/F 12/17	Prospective controlled	Plasma	Upregulation of nc-cfDNA and mt-cfDNA in rCD vs H. (no good markers for clinical remission, but no evidence of mucosal healing) Decrease plasma DNase activity in Males vs Females in rCD	[17]
8	Investigate whether IL-22 could beneficially modulate cellular function and epithelial gut homeostasis in situations of defective autophagy or ER stress.	1	7	0	Observational study (case-control)	Colonic biopsies	Upregulation of 'ISG score', IL-22, TNF and MLKL mRNA in aCD vs rCD. IL-22 mRNA positively correlated with colonic ISG score and IBD activity. IL-22 – ISG axis might be involved in therapy response by anti-TNF treatment in aCD.	[51]

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30 Age 37 [27–44] F/M 17/13	Investigate whether mtDNA is released during active disease, serving as a key pro-inflammatory factor in IBD	NA	NA	40 Age 33 [27–46] M/F 23/17	Prospective controlled	Plasma Stools Colonic resections	Upregulation of plasma mt-cfDNA and mt-peptides, fecal mt-DNA and colonic LP TLR-9 protein in CD vs H.	[57]
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Footnote: aCD: active Crohn's Disease; rCD: CD in remission; H: healthy controls; EV: extracellular vesicles; cfDNA: circulating free DNA. All cited studies received approval of the respective Institutional Ethical Boards, and are completed. Gong *et al.*, 2022: meta-analysis of CD colonic samples from NCT00639821ⁱ; the microarray data are available at the GEO accession number GSE16879^{iv}; GSE52746^{vi}; GSE59071^{vii}; GSE75214^{ix}. Wang 2021: publicly available RNA-seq and microbiome data on human IBD are part of the Integrative Human Microbiome Project (iHMP), IBD Multi'omics database IBDMDB^{xi}.

cGAS-STING DNA-sensing in Inflammatory Bowel Diseases

Outstanding Questions

- Intestinal mucosal damage, cell death and DNA release are hallmarks of IBD, and evidence of DNA sensing cGAS-STING pathway upregulation in the first IBD clinical studies warrant further mechanistic investigations to understand their contribution to disease evolution and potentially develop new therapeutic options.
- The difference in pediatric-onset IBD vs adult-onset IBD epidemiology and phenotype, raising with more aggressive and extensive features, remain largely unexplained, and characterizing the involvement DNA sensing and cGAS-STING pathway in colonic IBD pathophysiology may improve understanding.
- How can normal versus excessive concentrations of ectopic dsDNA be defined? Does cGAS interact with naked dsDNA, complex dsDNA-histones, or both? What are the functions of membrane and nuclear cGAS in colitis? Is cGAMP transferred to neighbouring cells by gap junctions in colitis as shown in vitro? Is there a link between IL-10 and AHR-dependent anti-inflammatory activity with STING activity in colitis?
- Further, investigating the role in IBD of 1) post-translational modifications in colitis beyond ubiquitination, 2) microbiota metabolites including short-chain fatty acids (SCFAs) such as butyrate, and 3) epigenetic changes such as DNA methylation, non-coding RNAs, and histone modifications, should identify potential new targets for therapeutic intervention to alleviate colitis symptoms.

Figure 1

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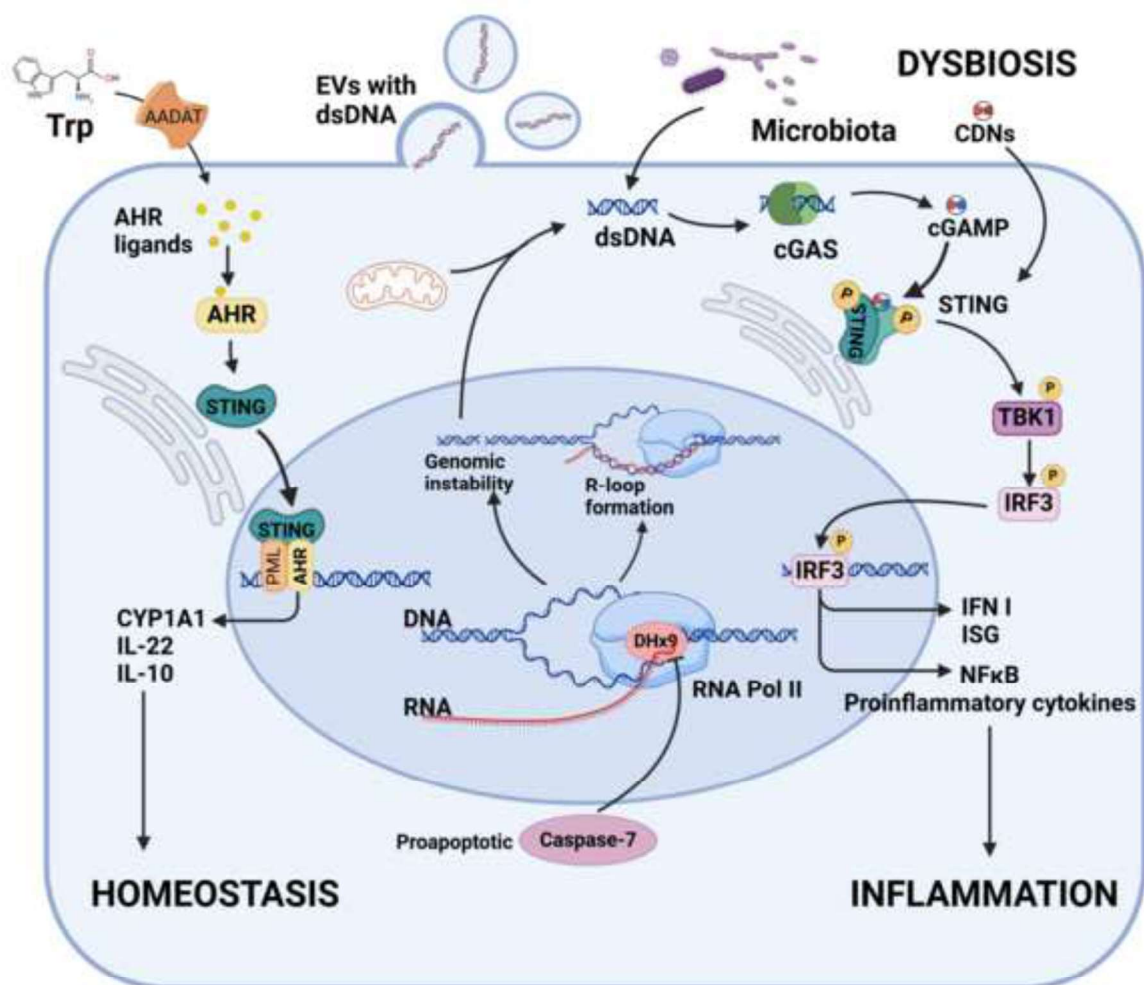


Figure 2

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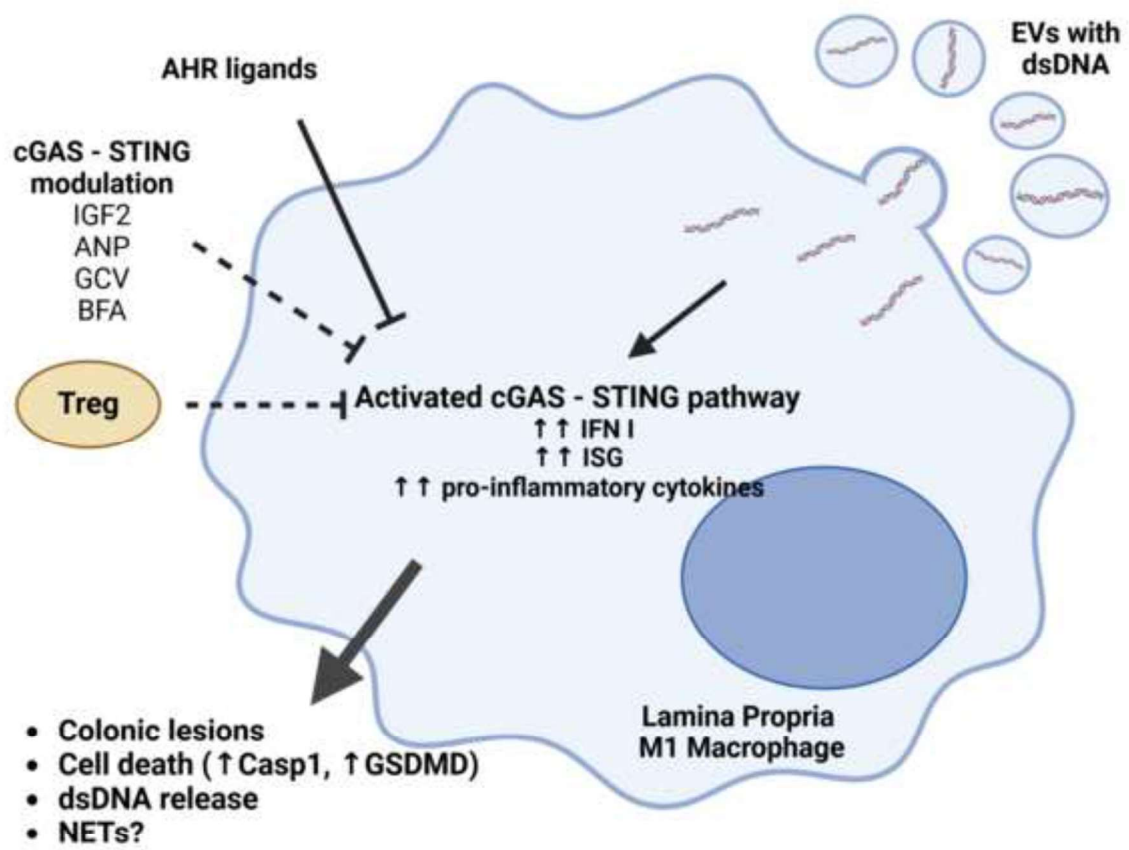


Figure 3

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