Antibiotics in the pathogenesis of diabetes and inflammatory diseases of the gastrointestinal tract

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Abstract

Antibiotic use is increasing worldwide. However, the use of antibiotics is clearly associated with changes in gut microbiome composition and function, and perturbations have been identified as potential environmental risk factors for chronic inflammatory disorders of the gastrointestinal tract. In this Review, we examine the association between the use of antibiotics and the onset and development of both type 1 and type 2 diabetes, inflammatory bowel disease, including ulcerative colitis and Crohn's disease, as well as coeliac disease and eosinophilic oesophagitis. We discuss the key findings of epidemiological studies, provide mechanistic insights into the pathways by which the gut microbiota might contribute to these diseases, and assess clinical trials investigating the effects of antibiotics. Such studies indicate that antibiotic exposures, varying in type, timing and dosage, could explain differences in disease risk. There seems to be a critical window in early life in which perturbation of the microbiome has a substantial effect on disease development. Identifying the antibiotic-perturbed gut microbiota as a factor that contributes to the pathophysiology of these inflammatory disorders might stimulate new approaches to prevention, diagnosis and treatment.

The gastrointestinal tract is subject to important chronic inflammatory diseases. These include diseases that affect the wall of the gastrointestinal tract, such as inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, as well as coeliac

disease and eosinophilic oesophagitis (EoE) (BOX 1). In addition, the pancreas is subject to inflammatory processes that can lead to either type 1 or type 2 diabetes. In this Review, we consider the relationship between these diseases and the gut microbiome, especially with respect to how antibiotic treatment for other indications can perturb the microbiome and affect the risk and course of these illnesses.

Humans, like other mammals, develop in a uterus that is routinely sterile or only occasionally visited by adventitious microbial pathogens[1,2]. The major exposure of the baby to the world of microorganisms occurs after rupture of the membranes and its descent through the birth canal and exposure to maternal vaginal and faecal microorganisms[1,2]. This is followed by successional colonization and blooms of taxa in the intestine that are highly conserved across all healthy infants[3-5]. The most dynamic period for the human microbiome is the first 3 years of life[6], which is also the period in which immunity, metabolism and cognition become well established. Studies in animal models have shown that perturbing the early-life microbiome, even transiently, can have long-term effects on these crucial developmental steps[7-12]. This conserved biology and the effects of experimental perturbation have led to the theory that an altered microbiota underlies a number of the diseases that are currently epidemic globally[13,14], including inflammatory conditions affecting the gastrointestinal tract.

Studies have shown that visceral, inflammatory and neuropathic pain can be influenced by the gut microbiome, which is particularly relevant in conditions in which pain can be a prominent symptom, including IBD and coeliac disease[15,16]. Inflammatory foot pad pain induced by carrageenan, `lipopolysaccharide` (LPS), tumour necrosis factor (TNF), IL-1β, and the chemokine CXCL1 in conventional mice was reduced in `germ-free mice`[17]. Similar experiments have demonstrated that mice develop visceral hypersensitivity following dextran sulfate sodium (DSS)-induced colitis, even after the intestinal inflammation has resolved[18]. This hypersensitivity was transferable by transplantation of a post-inflammatory microbiome to mice that had never been exposed to DSS but not by transplanting a control microbiome[18]. These findings provide evidence that the gut microbiota in mice contributes to the development of inflammatory hypernociception[18,19]. Specific data on the effect of the gut microbiome on pain in patients with IBD and coeliac disease are preliminary. In a pilot study of 21 children with coeliac disease, significant differences in relative abundance of specific bacterial taxa were associated with symptoms including abdominal pain[20], but further studies are warranted.

Antibiotics entered the general practice of medicine in the late 1940s and have since become pillars of modern medicine. Consequently, their use has steadily grown, and health practitioners increasingly rely on them. Estimated use exceeds one course per year for every person worldwide[21], and the numbers are growing. There is extensive variation in antibiotic use within localities, regions and countries, reflecting important differences in the culture of medicine and personal characteristics of both patients and practitioners[22]. Antibiotics also vary considerably in their antimicrobial spectrum of activity[23]. Antibiotics were developed to treat infections caused by bacterial pathogens, which has been the major thrust of both their development and their use. However, when an antibiotic is taken, it also has collateral effects on the resident microbiota: inhibiting some, and thus reciprocally selecting for

others. In addition to selecting for potential pathogens within the microbiome, including *Staphylococcus aureus* and *Clostridioides difficile*, antibiotics considerably perturb the human gut microbiome, with effects lasting for months or longer[24-26]. In the past, it was widely assumed that after a course of antibiotics the 'normal flora' would bounce back to its pretreatment state. Unfortunately, using molecular tools that have greater precision than culture-based studies, it has become clear that the microbiome is perturbed for months, and might never resume to its pretreatment state[24-26]. This finding is especially important for young children, in whom the microbial succession is highly choreographed and perturbations, even if transient, can affect both microbiome and host development[3-5,8,25].

In mixed microbial populations, such as the gut microbiome, fungal numbers usually increase after exposure to antibiotics[27]. Many gut commensal fungi, including *Candida* species, interact with host epithelial and immune cells[28,29]. The host adaptive immune response is targeted to hyphal cells[29], and thus any shift in the balance between yeasts and hyphae will affect the immunological milieu. Such phenomena could contribute to antibiotic-induced exacerbations of both disease predisposition and the disease itself.

As such, there is growing interest in the hypothesis that owing to their effects on the gut microbiome, antibiotic use might have unintended collateral clinical consequences, especially when given to young children, whose developing microbiome is both plastic and interlinked with host development[30]. In this Review, we consider this hypothesis in the context of several chronic inflammatory diseases that affect the human gastrointestinal tract and that have increased in incidence during the antibiotic era (FIG. 1).

Diabetes

Approximately 537 million people worldwide have either type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM)[31]. Epidemiological studies of the incidence and prevalence of T1DM and T2DM in 212 countries/regions have been extensively reviewed by the International Diabetes Federation and published in the Diabetes Atlas[31]. Although hyperglycaemia is the common element in T1DM and T2DM, the diseases differ extensively in epidemiology and pathogenesis, and are therefore considered separately.

Type 1 diabetes

Epidemiology

An important factor in T1DM is the genetic predisposition provided by specific HLA haplotypes, mainly DR3-DQ2 and DR4-DQ8 (REF.[32]). However, as the age of onset is swiftly decreasing, these predisposing genes cannot solely explain the rapidly rising incidence of T1DM worldwide[32-34]. Altered gut microbiome composition (referred to as dysbiosis) has been identified as a potential environmental risk factor[35]. The gut microbiota of patients with T1DM harbour a lower ratio of Firmicutes to Bacteroidetes, have decreased *Bifidobacterium* spp. abundance, reduced bacterial richness and diversity, and lower production of short-chain fatty acids (SCFAs) compared with healthy individuals[36-39]. Although such changes are seen largely in children already affected by the disease, some were already present before clinical onset.

Changing epidemiology of T1DM in the antibiotic era.—In the past few decades, the worldwide incidence of T1DM has risen dramatically, particularly in children under 14 years old[31,40]. The estimated annual global number of newly diagnosed children rose by approximately 50% from 65,000 in 2003 (REF.[41]) to 98,300 in 2021 (REF.[31]), a 3% annual increase. In 2021, >1.2 million children and adolescents worldwide had T1DM[31]. However, there is striking geographic variation in the reported incidence of T1DM, with the highest annual incidence reported in children in Europe (~31,000 cases (5.26% of all children in Europe)) and the lowest in children in the Western Pacific (~11,600 cases (1.88% of all children in the Western Pacific))[31]. Discrepancies between regions must be interpreted with caution, as data sources on T1DM incidence in low-income regions are scarce.

Epidemiological linkages with the disease.—The exact causes of the steep increases in incidence of T1DM are not yet known[42]. Rapid changes within a short span of time are more likely a result of changes in environmental risk factors than changes in genetic risk[32]. Intriguingly, the rising incidence of T1DM in children began in many countries/regions in the middle of the twentieth century, coinciding with the start of the antibiotic era[43]. For example, the rise in Finland preceded the widespread introduction of antibiotics, which is consistent with changes in sanitation, such as the use of chlorinated drinking water. These improved hygiene conditions led to reduced exposure to infectious agents in early childhood. This 'hygiene hypothesis' is supported by the negative correlation between hygiene conditions and T1DM incidence[44].

Two important changes in medical practice in the second half of the twentieth century were antibiotics and the increased frequency of caesarean section. Antibiotic use in early life clearly leads to changes in the intestinal microbiome[3,45]. Similarly, children born by caesarean section begin life with an altered microbiome[3,5,46,47], and the changes, including reduced *Bacteroides* species, and altered community composition can persist throughout the first year of life[3,46-48]. Birth by caesarean section is usually a compounded insult to normal microbiome development, involving loss of the natural passage through the birth canal, and the administration of high doses of antibiotics to the mother in the peripartum period[49]. Children born by caesarean section also seem to be more likely to receive antibiotics in early life[50,51] (TABLE 1). Antibiotics are widely administered to children on the basis of the clinical premise of important benefit and minimal risk; however, antibiotic overuse is well documented in children as well as in older people[52,53], and prescribing rates vary widely with differences between countries/regions (both high-income and low-income to middle-income countries) as well as regional differences, even among children with similar clinical presentations[22]. Parallel statements can be made about caesarean section[49]. Currently, only limited data on the association between the risk of T1DM and the use of antibiotics are available, mostly provided by Scandinavian nationwide cohort studies[50,51,54,55] (TABLE 1), where most children are exposed to antibiotics in early life.

Two longitudinal cohort studies from Sweden and Denmark found an increased risk of T1DM after antibiotic exposure in early life[50,51], However, mode of delivery is a strong confounder, as a larger effect was observed in children delivered by caesarean section compared with those delivered vaginally. Although similar in the magnitude of the increased risk ratio, two other cohort studies from Denmark and Norway found no

significant association between the use of antibiotics and T1DM onset, irrespective of antimicrobial spectrum or use in an age-specific period[54,55] (TABLE 1). These results might reflect the differences between countries/regions, types of antibiotics used, and exposure to `probiotics`, among other factors. Such variation might lead to non-significant associations. More homogeneous cohorts, with varying ethnicities and geographical regions, will better assess whether perturbation of gut microbiome composition as a result of caesarean section and/or antibiotic exposure in early life influences the onset of T1DM.

Experimental studies

The composition of the intestinal microbiota in early life has a large effect on immunological development in both intestinal and systemic sites[10,11,56-59]. Therefore, perturbations of microbiome composition during this critical window might have a key role in T1DM onset, which is shown by studies using `non-obese diabetic mice` (NOD), an experimental model resembling T1DM in humans[60]. The variation in T1DM incidence in these NOD mice is dependent on the composition of the microbiome to which the mice are exposed[61-63]. A general rule of thumb is that 'dirty protects'; NOD mice reared in ultra-clean facilities develop T1DM at higher rates than those in more standard facilities[64]. As such, germ-free mice are more prone to develop T1DM than NOD mice exposed to a single bacterium[62,63]. NOD mice with deficient innate immunity owing to a null mutation of the Toll-like receptor adapter signalling molecule MYD88 are protected from T1DM development under specific-pathogen-free conditions, but not under germ-free conditions[65], indicating that the microbiota signal is transduced through MYD88. A particular taxon, the genus *Candidatus* Savagella (formerly known as segmented filamentous bacteria (SFB)), protected NOD mice against T1DM by inducing small intestinal T_H17 cell populations[63]. These results indicate an important and complex interplay between the microbiota and immunological effectors in T1DM (FIG. 2).

Timing and nature of antibiotic exposure.—As specific types of antibiotics exert differential effects on gut microbiota composition, exposure to particular antibiotic classes can differentially affect T1DM development[66-68]. Timing of antibiotic exposure is also a potential factor[66,69]. In NOD/Caj mice, a substrain of NOD mice used to understand the role of B cells as antigen-presenting cells, maternal (prenatal) exposure to neomycin or vancomycin both induced long-term changes in the gut microbiome composition of the offspring compared with the offspring of untreated control mice[66]. However, only vancomycin (which targets mainly Gram-positive bacteria and anaerobes) strongly accelerated T1DM development[66]. In mice prenatally treated with vancomycin, T cells from the spleen, pancreatic lymph nodes and Peyer's patches showed significant decreases in naive T cell markers ($CD44^-CD62L^+$) and increased numbers of $CD4^+$ memory T cells ($CD44^+CD62L^-$). Consistent with this more-activated T cell repertoire, these T cells expressed higher levels of the pro-inflammatory cytokines IL-17, interferon-γ (IFNγ) and TNF. By contrast, prenatal treatment with neomycin (which targets mainly aerobic microorganisms) significantly protected the progeny from T1DM compared with untreated counterparts, and was associated with increased Bacteroidetes abundance[66]. The protection was also associated with induced immunotolerogenic responses of antigen-presenting cells in both spleen and mesenteric lymph nodes[66].

In another study, lifelong treatment of NOD mice with either vancomycin or neomycin, started prenatally until the onset of diabetes, accelerated T1DM onset and altered effector T cell populations, with increased IFNγ CD4^{+} T cells and, in contrast to the previously discussed study[66], reduced IL-17^{+}CD4^{+} T cells[70]. In male but not female NOD mice treated with a broad-spectrum high-dose antibiotic cocktail (streptomycin, colistin and ampicillin) or vancomycin only, T1DM incidence was significantly increased compared with the incidence in untreated control mice[68] ($P < 0.0001$ for the antibiotic cocktail and $P < 0.0004$ for vancomycin) and showed a significant decrease in *Il17a* gene expression or IL-17-producing cells in colon, Peyer's patches and mesenteric lymph nodes[68]. However, another study showed that T1DM was attenuated in NOD mice that received vancomycin from birth through weaning at age 4 weeks[69]. These conflicting results might reflect the nature of the microbiota in the mouse colony being studied, but nevertheless they provide experimental evidence that antibiotic perturbation of the microbiome affects T1DM development.

Most studies investigating the effects of antibiotics in murine models have used a continuous antibiotic regimen, often at super-therapeutic levels[67,68]. However, such interventions do not mimic paediatric antibiotic use, which consists of discrete courses that are modelled better by therapeutic-dose pulsed antibiotic treatment (PAT). A study in which NOD mice received PAT (with a macrolide) in early life showed accelerated development of T1DM and insulitis compared with mice continuously treated with subtherapeutic antibiotic treatment (STAT) and controls[71]. Male mice exposed to PAT showed reduced α-diversity and β-diversity in their microbial population structure and decreased proportions of small intestinal lamina propria T_H17 and regulatory T (T_{reg}) cells before T1DM onset. These immunological changes were accompanied by altered ileal gene expression (concomitant with upregulated cholesterol biosynthesis) and altered metabolomic profiles in the caecum, liver and serum[71]. Transfer of the antibiotic-altered microbiota to adult germ-free mice showed similar changes in intestinal T cell populations, confirming that the perturbed microbiome was responsible for the altered immunological signal. However, transfer of antibiotic-perturbed microbiota from 6-week-old mice to pregnant germ-free mice showed an unexpected protection of the offspring from T1DM[71]. One potential explanation for this observation is that the 6-week time point (P42) microbiota were highly selected for opportunistic microorganisms, and their transfer to the newborn mice led to tolerance, consistent with the adage in NOD mice that 'dirty protects'. This complexity, shown in a single series of experiments by the same group[9,72], illustrates that the relationship between the microbiota and host phenotypes depends on antibiotic type, dosage and timing. In independent experiments, even a single 5-day macrolide PAT course in early life was sufficient to accelerate and enhance T1DM onset in male mice, leading to profound changes in expression of genes encoding immunological effectors in the ileum[72]. A study published in 2021 explored whether the phenotype of mice that had been exposed to antibiotics that perturbed the microbiome, changed immunological phenotypes and accelerated and enhanced T1DM, can be rescued by attempting to restore their microbiota[73]. To investigate this, during a period between 3 and 7 days after antibiotic treatment ended, mouse pups were gavaged with caecal microbiota of mothers obtained on the day of their pups' birth. This treatment largely restored the baseline T1DM phenotype, partially restored the intestinal microbiome composition, metagenome and metabolome, and

restored ileal RNA and microRNA expression. These studies demonstrate the importance of the effect of antibiotic perturbation of the microbiome on T1DM development, and point towards the role of post-exposure restorative approaches. They also provide a path to discovery of relevant microorganisms, microbial genes, metabolites and host genes that influence the propensity for T1DM[73].

Clinical trials

An intervention study that used the antimicrobial fusidic acid in 28 patients with newly diagnosed T1DM showed no significant differences in β-cell function, C-peptide values or quantitative insulin requirements compared with a control group who received placebo[74]. In addition, a clinical trial with an oral dose of the SCFA butyrate showed no effect on either innate or adaptive immunity markers in 30 patients with long-standing T1DM[74,75]. By contrast, in a pilot trial including 20 patients with recent T1DM onset, use of faecal microbiota transplantation (FMT) led to an increased abundance of both *Desulfovibrio* strains and microbiota-derived plasma metabolites of tryptophan origin, which were associated with the stabilization of residual β-cell function[76]. This result provides a proof-of-principle that even after T1DM commencement, interventions affecting gut microbiome composition and activity can have salutary effects, extending the findings observed in mice. Taken together, such findings warrant added caution in the use of antibiotics in pregnant women and newborns, and minimizing the practice of caesarean section.

Type 2 diabetes and childhood obesity

Epidemiology

Changing epidemiology of the disease in the antibiotic era.—In the USA, the prevalence of T2DM has increased from 0.93% in 1958 to 7.40% in 2015 (REF.[77]). This steep rise coincides with an increased prevalence of obesity, one of the hallmarks of T2DM, as well as with the cumulative and increasing use of antibiotics[52,53,78]. The worldwide prevalence of T2DM is estimated to increase to 12.2% by 2045 (REF.[31]).

Metagenomic analysis of the gut microbiome of patients with T2DM revealed distinct perturbations in composition and function, characterized by a decreased abundance of butyrate-producing bacteria and an enrichment of opportunistic pathogens, often mucin-degrading (*Akkermansia muciniphila*) and sulfate-reducing *(Desulfovibrio* sp.)[79]. Although some studies showed an increased Firmicutes to Bacteroidetes ratio in patients with T2DM[80,81], this should not be considered a T2DM hallmark as the relative abundance of these phyla is highly variable between individuals with T2DM[82,83]. These compositional changes are partly explained by differences in ethnicity; lower α-diversity was observed in the gut microbiomes of populations of South Asian and African origin compared with those of European origin living in the same city[84,85]. Such differences correspond to a higher risk of T2DM in these ethnic minority populations[86].

Epidemiological linkages with the disease.—As T2DM mostly affects adults, studies examining the association between antibiotic use and T2DM diagnosis have only been performed in adult cohorts[87-89] (TABLE 1). However, multiple longitudinal cohort studies

have shown that exposure to antibiotics in the first 3 years of life is associated with an increased risk of childhood obesity and central adiposity, which are well-known risk factors for T2DM[48,90-95] (TABLE 1). Strikingly, these studies showed that most children (>70%) in high-income countries/regions are exposed to antibiotic therapy at least once by the age of 2 years[90], whereas the average incidence of antibiotic use in low-income and middle-income countries/regions is even higher (4.9 courses per child per year)[96]. This enormous use is at a scale consistent with the extent of disease incidence; antibiotic use in developing countries/ regions started later, but cumulatively they are catching up quite rapidly[97].

Antibiotic use during pregnancy also has effects on the development of the infant microbiome[98]. Exposure to antibiotics in the second or third trimester has been associated with an 84% (95% CI 33–154%) higher risk of childhood obesity, including higher waist circumference (3.13 cm, 95% CI 0.68–5.59 cm) and increased body fat percentage (1.86%, 95% CI 0.33–3.39%)[48]. Although no direct information on T2DM was provided in these childhood studies, these findings provide consistent evidence that exposure to antibiotics during the critical window in early life, even if brief, could lead to long-term effects that increase the risk of developing T2DM.

Adult cohort studies consistently show that antibiotic exposure at least 6 months before the date of diabetes diagnosis is associated with increased risk of T2DM (TABLE 1). The risk increases with more frequent antibiotic exposure[87-89] and varies depending on antibiotic type[88,89]. However, these studies vary in their methodological approaches. For example, not all studies adjusted the results for variables including obesity, dyslipidaemia, hypertension and other cardiovascular comorbidities[88], conditions that are part of the metabolic syndrome and are associated with T2DM as well as gut dysbiosis[99,100]. That the gut microbiome is influenced by ethnicity[84], as well as environmental factors such as diet, physical activity, smoking and other medications[101], limits the interpretation of epidemiological studies. As such, the causal link between antibiotic exposure and its subsequent perturbation of the gut microbiome, with T2DM development, has not been established by the epidemiological studies.

Experimental studies

Murine models and faecal transplant experiments have provided mechanistic insights into how the gut microbiota might contribute to T2DM development. In particular, they have shown that the intestinal microbiota influences both host metabolism and immunological interactions. Early-life STAT exposure in C57BL/6J mice, either continuously or for a short time span only, led to significantly increased fat mass compared with control mice[7,8,102,103], with increased incretin secretion and glucose intolerance compared with controls[7,8,103]. Antibiotic treatment also altered expression of hepatic and ileal genes involved in fatty acid metabolism and triglyceride uptake, as well as hepatic steatosis[9,102,103]. The gut microbiota of STAT mice showed a shift in taxonomic composition, with higher levels of Firmicutes and lower levels of Bacteroidetes[7,8,102], similar to that observed in ob/ob (leptin-deficient, obese) mice[99,104]. Importantly, the onset of adiposity occurred after the alterations in microbiome composition and remained later in life (32 weeks)[7,8], even after the perturbation of the microbiome recovered following STAT withdrawal[8,102]. Transferring

caecal contents from STAT mice into germ-free mice by oral gavage replicated the obesity phenotype[8]. These data are consistent with the hypothesis that there is a critical early-life period in which later-in-life metabolic development is set, and that alterations to the gut microbiota in that period have long-term consequences. Caecal contents of STAT mice showed substantially higher levels of SCFAs (acetate, butyrate and propionate), which indicates an increased capacity to harvest energy by bacterial fermentation of complex dietary carbohydrates[7]. SCFAs are ligands of the G protein-coupled receptors GPR41 and GPR43, which are expressed by intestinal enteroendocrine cells that produce peptide YY[105], a hormone that affects insulin utilization by increasing intestinal transit time and increases satiety and energy harvest from the diet. However, the role of GPR41 and GPR43 in obesity is inconsistent between studies. A study with GPR41-knockout mice showed that they have reduced body weight, less fat accumulation and less insulin resistance than their wild-type counterparts[106]. Another study showed that weight gain is suppressed in mice that overexpress GPR43 in white adipose tissue and that are fed a high-fat diet (HFD), whereas GPR43-knockout mice become obese, even on a normal diet[107]. These seemingly contradictory results might reflect factors such as differences in the disease models used, the inbred mouse strains used and their microbiota, or non-specific effects of the knockouts themselves[108]. Epithelial cell expression of GPR41 and GPR43 in human physiology needs more study. Nevertheless, these studies provide evidence that SCFAs, and therefore the gut microbiota, regulate host energy expenditure. As such, disruption of gut microbiome composition and functionality by antibiotic exposure affects host energy balance, at least in part.

High circulating levels of inflammatory effector molecules, including TNF, IL-6, IFNγ and bacterial LPS, have been consistently associated with both obesity and T2DM, providing a potential mechanism for the persistent, low-grade inflammatory state of liver, muscle and adipose tissue frequently observed in both obesity and T2DM[109-114]. These changes might reflect increased intestinal permeability, leading to translocation of microbial constituents and products into the systemic circulation[110]. Conventionally raised mice receiving continuous LPS infusions showed metabolic responses, including increased hepatic insulin resistance, similar to those in mice receiving a HFD[109]. In 6-week-old ob/ob mice characterized by high LPS levels, antibiotic treatment lowered plasma LPS levels and inflammatory markers in adipose tissue[110]. These changes occurred concomitantly with improved metabolic parameters (such as improved glucose tolerance, less weight gain and lower fat mass). Notably, similar beneficial effects on glucose metabolism, including improved glucose tolerance and reduced fasting glucose levels, were seen in lean, healthy male mice after different 4-week antibiotic regimens[115]. These improved metabolic processes were accompanied by changes in hepatic and ileal gene expression involving glucose regulation and bile metabolism. Thus, after perturbations that increase translocation of intestinal contents, antibiotic treatments reduce secondary effects, and are therefore useful tools for future experiments. However, early-life exposure to antibiotics also directly affects host immune phenotypes, as illustrated by altered CD4+ T cell subsets and reduced intestinal secretory IgA levels in mice exposed to antibiotics compared with conventionally raised mice[9]. Germ-free mice exposed to antibiotics did not exhibit any substantial immunological changes, indicating that the metabolic and immunological effects were not a direct result

of the antibiotics but rather are consequences of the antibiotic-induced gut microbiome alterations[9] (FIG. 2). Thus, perhaps paradoxically, although early-life antibiotic exposures can drive the altered pathophysiology, once the damage has been done (from whichever cause), antibiotics have the potential to improve outcomes.

Dietary intake is one of the key modifiable extrinsic factors that influences gut microbiome composition and contributes to the onset of both T2DM and obesity. Antibiotic exposure in early life aggravates the negative effects (which include dyslipidaemia, insulin resistance and increased visceral fat mass) that accompany a HFD in female BALB/c mice compared with mice similarly exposed to antibiotics but fed a normal diet, even when the dysbiosis progressively recovers[116]. In another study, mice fed an obesogenic diet showed increased weight and fat mass when receiving lifelong STAT compared with their unexposed HFD-fed counterparts[103]. Such an obesogenic diet also affects antibiotic susceptibility. HFD-fed mice had impaired efficacy of bactericidal antibiotics compared with normally fed mice, a difference that was not observed in microbiota-depleted animals[117]. These findings suggest that antibiotic exposure worsens the diet-induced adiposity phenotype, leading to increased T2DM risk, and that obesity also reduces antimicrobial susceptibility.

Clinical trials

Owing to concerns about antibiotic resistance, there are few clinical trials that have investigated the effects of antibiotic exposure on weight gain in children. The trials that have been performed show conflicting results, with early-life exposure to antibiotics either having pronounced effects on weight gain[118] or no effect[119]. A meta-analysis of ten randomized controlled trials including 4,316 children showed that undernourished children (<12 years old) from low-income and middle-income countries/regions treated with antibiotics were significantly taller (0.04 cm/month, 95% CI 0.00–0.07 cm/month) and had increased weight gain (23.8 g/month, 95% CI 4.3–43.3 g/month) compared with their placebo (nine studies) or untreated (one study) control groups[117]. However, there was significant geographic variation in weight gain, with children from trials conducted in Africa gaining 35.6 g/month (95% CI 12.8–58.3 g/month) of body weight more than children from other regions, which possibly is a reflection of more severe malnutrition[118]. In a US trial, 302 children (<6 years old) taking long-term oral trimethoprim–sulfamethoxazole prophylaxis did not gain substantially more weight than 305 control individuals, although approximately 25% of children in both groups had overweight or obesity[119].

Studies of antibiotic interventions to alter T2DM progression have been limited in number. Treatment with a broad-spectrum antibiotic mixture in healthy lean men led to a drastic reduction in the abundance of gut microbiota (in colony-forming units (CFU)), but without significant changes in fasting or postprandial glucose, insulin secretion or plasma lipid levels[120]. Similar effects on gut microbiota composition were seen after oral vancomycin therapy[121,122]. Bacterial diversity was significantly ($P < 0.001$) reduced, with lower abundance of Gram-positive bacteria and increased abundance of Gram-negative bacteria, concomitant with increased levels of circulating LPS[121,122]. These perturbations affected glucose metabolism by decreasing peripheral insulin sensitivity in both lean individuals and patients with metabolic syndrome. In another study, 48 patients with bacterial endocarditis

treated with intravenous vancomycin and gentamicin showed substantially increased BMI ($+2.3 \pm 0.9$ kg/m^2), which persisted 1 year after treatment[123]. However, as the therapy improved clinical status, weight gain could have been a reflection of the overall health improvement.

Novel therapies that aim to restore the dysbiotic gut microbiota in individuals with obesity and T2DM have been investigated in humans using different `prebiotic`[124], probiotic[125] or `synbiotic`[126] regimens, but beneficial effects have been elusive. FMT has been studied as a means of counteracting the dysbiotic gut microbiome to improve insulin sensitivity in patients with metabolic syndrome or T2DM. Transfer of faeces from healthy donors to patients with metabolic syndrome improved insulin sensitivity in some, but not in all, patients[127,128]. This dichotomy might reflect baseline intestinal bacterial differences, with responders having lower diversity before FMT[128], or differences in donor FMT composition and its administration[129].

Inflammatory bowel disease

Definitions and epidemiology of IBD

IBD is a chronic inflammatory condition of the intestines, with two main subtypes: ulcerative colitis and Crohn's disease. Although the precise aetiology of IBD remains unknown, increasing data suggest that alterations in the intestinal microbiota are a major contributor to IBD risk. As such, antibiotic exposure might have a role in IBD development in the current era.

One feature of IBD that might help in understanding its underlying pathophysiology is its temporal variation. Not only in disease activity, extent or behaviour within a patient, but also in epidemiological features that have evolved globally over time. Although the disease initially seemed to mostly be limited to Northern and Western Europe, the USA and Canada, data from 1960–2017 show that IBD has become increasingly prevalent worldwide, including in Central and South America, Africa, and the Caribbean and Asia–Pacific regions[130-132]. Furthermore, incidence in newly identified hotspots might outpace that of regions with traditionally high prevalence[130,131], which is consistent with the rising cumulative exposure to antibiotics in those areas.

The demographics of those with IBD have also evolved. Ulcerative colitis is the predominant IBD subtype in regions with newly-incident IBD, and Crohn's disease incidence then increases over time[130]. Countries/regions with traditionally high prevalence tend to have similar distributions of Crohn's disease and ulcerative colitis[130]. Among the 34 countries/regions in the Organisation for Economic Co-operation and Development (OECD), IBD hospitalizations (a proxy for disease severity) were highest in traditionally high-prevalence regions (North America, Europe and Oceania) and lowest, but also most rapidly increasing, in new-incidence regions (Asia, Latin America and the Caribbean)[133]. In the Asia–Pacific Crohn's and Colitis Epidemiology Study (ACCESS) inception cohort of 413 patients (181 with Crohn's disease, 222 with ulcerative colitis and 10 with unclassified IBD), ~20% of patients with Crohn's disease who initially presented with a non-fistulizing, non-stricturing phenotype developed these complications after a median follow-up of 18 months[134], which

is similar to rates in Western populations[135]. However, patients with ulcerative colitis in the ACCESS cohort were less likely to need advanced medical therapies and also less likely to have colectomies compared with their counterparts with Crohn's disease, as well as in comparison to patients with ulcerative colitis in Western populations[134,136].

Although the time of IBD onset can span from infancy to older age, diagnoses occur most commonly around the third decade of life[132,137]. However, there has been an increase in paediatric-onset IBD incidence in the past few decades, particularly in children aged <10 years[137-139], in countries/regions with a high prevalence of IBD. For example, a population cohort study in Ontario, Canada, found an annual increase of 9.7% from 1999 to 2008, although this might have reflected improved early diagnosis[137]. However, IBD incidence increases following immigration from a low-prevalence to a high-prevalence country/region, with stronger risk associated with younger age at time of immigration (similar to T1DM) and among subsequent generations born in the high-prevalence countries/regions[140-142]. Such studies provide direct evidence of the importance of environmental factors in IBD risk. Although genome-wide association studies[143,144] and twin studies[145,146] have clearly identified genetic contributors to IBD risk, the changes in global IBD patterns and the time frame in which they have occurred emphasize the critical role of environmental triggers. Notably, many of the environmental factors identified as being associated with IBD risk or protection (for example, migration, diet and breastfeeding)[145,146] are related to changes in the intestinal microbiome[147-149].

Antibiotic exposure and IBD risk in humans

Several large cohort studies have implicated the use of antibiotics in the risk of IBD. Two large national database studies, one from the UK[150] (a registry of 1,072,426 children aged <18 years with 6.6 million person-years of follow-up from 1994 to 2009) and another from Denmark[151] (a registry of 577,627 children with 3,173,117 person-years of follow-up from 1995 to 2003), both found that antibiotic exposure in childhood is associated with IBD risk in a dose-dependent manner. This relationship has been confirmed in both paediatric-onset and adult-onset IBD (with approximate odds ratios ranging from 1.3 to 3.4), although the risk is higher with childhood exposure, with the greatest quantity of evidence and strength of effect in children exposed to antibiotics before the age of 1 year[150-157]. Although some studies have found associations between antibiotic treatment and subsequent development of both Crohn's disease and ulcerative colitis[152,154], in other studies the relationship was stronger either for Crohn's disease (that is, significant findings at lower exposure levels)[150] or present for Crohn's disease only[151,155,158]. However, many of these studies were conducted in children, and the distribution of Crohn's disease compared with ulcerative colitis, as well as the primary drivers of disease aetiology, may differ from those in adults[158]. For example, although the increasing incidence of very early onset IBD in Canada (that is, onset before age 6 years)[159] suggests that its aetiology contains an environmental component, it is more commonly associated with monogenic mutations than IBD that starts later in childhood or in adulthood[160]. A potentially important confounding factor is the nature and severity of the illnesses for which antibiotics are prescribed. One hypothesis is that antibiotic exposure is merely a proxy for the underlying acute infections, which themselves might be the primary factor for increased risk of IBD, or alternatively might be

a protective factor against IBD (the 'hygiene hypothesis'). Unsurprisingly, such issues are difficult to tease out from studies in children[161], which is why models in experimentally exposed animals, in the absence of initiating infections, are useful.

Animal models of antibiotics in IBD

Given the lag between antibiotic exposure and IBD onset, as well as potential clinical confounders associated with antibiotic use, animal studies have been particularly helpful in establishing causal relationships between antibiotic treatment and changes in intestinal inflammation in IBD models. IL-10-deficient[162] and SAMP1/YitFc[163] mice spontaneously develop bowel inflammation, and disease activity is responsive to microbiome manipulations[164,165]. Studies in both models have shown that antibiotic treatment improves intestinal inflammation when used in either preventive or treatment strategies[165,166]. Studies in the IL-10-deficient model demonstrated that particular antibiotic classes improve colitis in different intestinal regions, suggesting that specific microbiome populations have roles in modulating intestinal inflammation[166]. In experiments that model how vertical transmission of the maternal human microbiome to infants might affect IBD development, Schulfer et al. gavaged gnotobiotic IL-10-deficient mouse dams with faecal microbiota from wild-type mice that had either been perturbed by antibiotics administered at weaning in their drinking water or had not (control microbiota)[167]. In the absence of any other intervention, pups born to dams gavaged with the antibiotic-perturbed microbiota developed substantially more severe colitis than those born to dams given the control microbiota. In this experiment, the fact that neither the pups nor their mothers were actually exposed to any antibiotic established that the antibiotic-perturbed microbiota passed down from the mothers was sufficient for the enhanced disease. Parenthetically, this experiment also provides evidence that in diseases with familial tendencies, the risk factors might not be only the inherited host genes, but also the intergenerational transfer of microorganisms. Similar experiments were conducted by Miyoshi et al. with similar results[168]. Taken together, these studies provide an important link between cumulative antibiotic use in populations and the increasing IBD disease risks observed over the past few decades. They are consistent with the notion that antibiotic effects are cumulative across generations, as previously postulated[169]. Studies in humans investigating the effect of inheriting a perturbed microbiome on IBD risk are ongoing[170].

Another important experimental model of IBD is DSS-induced colitis[171,172]. Ozkul and colleagues[173] investigated whether previous exposure to antibiotics worsened the course of DSS colitis. They observed that mice with early-life exposure to a macrolide develop more severe colitis than mice without the exposure. That the antibiotic exposure ended more than 2 weeks before the DSS challenge indicates potential latency in antibiotic effects, which is consistent with observations in children[153]. In a subsequent experiment, the investigators transferred antibiotic-perturbed microbiota obtained 30 days after the exposure ended to germ-free mice, who were subsequently challenged with DSS; the recipients of the antibiotic-perturbed microbiota developed more severe colitis than those given the normal microbiota. Taken together, these studies suggest that antibiotic perturbation of the microbiota, in the absence of any infection, worsens experimental models of colitis. In other experiments in which the antibiotic challenge preceded exposure to a colonic pathogen of

mice (*Citrobacter rodentium*) by as much as 80 days, there was worsened inflammation compared with mice not exposed to antibiotics[174]; the antibiotic-perturbed microbiota also transmitted more severe disease to germ-free mice that had been conventionalized, indicating that the perturbed microbiota per se had pathogenic properties[174].

Antibiotics in the treatment of IBD

A separate question is whether antibiotics can modulate disease activity in established IBD, as with diabetes. There has been a long-established, but under-studied, clinical practice of using antibiotics in treating complications of IBD, such as fistulae, abscesses and pouchitis[175-178]. A related question is whether antibiotics can be used more routinely to alter the natural history of IBD. However, randomized controlled trials (and related meta-analyses) have yielded conflicting results[179,180] (TABLE 2). The literature is difficult to interpret given the diversity of antibiotics studied as well as the differing indications and timing of treatment. The end points for some of these trials were based on clinical symptoms, and were therefore subject to potential confounding by treatment of irritable bowel syndrome, a condition that frequently co-exists with IBD[181] and for which antibiotic treatment can have some efficacy[182]. Nonetheless, one of the clearest examples of the utility of antibiotics in the management of IBD is the use of nitroimidazoles, specifically metronidazole and ornidazole, to prevent postoperative recurrences of Crohn's disease. Compared with placebo, both medications reduced (by 25–30%) the proportion of patients with endoscopic recurrence 3 months after surgery[183,184]. In the setting of defined clinical interventions, such as surgery, antibiotics can clearly improve outcomes.

Beyond the direct effect of antibiotic-perturbed microbiota on IBD risk and activity, antibiotics might also influence disease by altering the metabolism of IBD medications by the gut microbiota[185,186] (FIGS. 2,3). Sulfasalazine, which was among the earliest recognized IBD treatments, comprises an anti-inflammatory 5-aminosalicylate (such as mesalamine) joined to the antimicrobial sulfapyridine via a diazo bond. The cleavage of this bond, and the subsequent release of the active moiety, is mediated by diazo reductases, which are produced by many gut bacterial taxa but in the largest quantity by Clostridia[186,187]. Notably, antibiotic-treated germ-free rats do not excrete cleaved sulfasalazine[186,187]. The gut microbiota also has roles in metabolism of other IBD medications including glucocorticoids[188], methotrexate[189] and thioguanine[190]. Thus, antibiotic-induced microbiome manipulation might affect established IBD in addition to having effects on disease development.

FMT as an approach for treating IBD

The growing interest in FMT, coupled with high-throughput sequencing, permits a new understanding of the interactions between the intestinal microbiota and IBD activity. Despite differing delivery routes and treatment regimens, three of the four randomized clinical trials of FMT for the treatment of mildly to moderately active ulcerative colitis yielded remission rates of ~25–30% compared with 5–10% in controls[191-194]. In a pilot, randomized, controlled study of 12 patients with mild-to-moderate ulcerative colitis published in 2021, remission was numerically more common in the treatment group but was not significantly different between the treatment group and the control group (two of six versus none of

six, respectively)[195]. Another negative study, published in 2015, evaluated 50 patients who were randomized to either two donor FMTs over 3 weeks by nasoduodenal tube or placebo (autologous FMT). The primary end point evaluation at 12 weeks was completed by 37 patients. In both the per-protocol and intention-to-treat analyses, there were no significant differences between groups, although some have attributed the negative result to the less-intensive dosing interval compared with the interval used in the positive FMT trials[192]. Across trials, consistent taxa or mechanisms underpinning the successful FMTs have not been identified. Nevertheless, the proof of principle underlying these trials is promising, and has spurred development of defined microbial consortia instead of stool. Although there have been a few open-label studies and case reports of FMT for Crohn's disease that have had positive results[196], the only two randomized clinical trials to date did not show significant effects[197,198]. However, data interpretation is limited by heterogeneity in disease location, behaviour and extent, which makes Crohn's disease difficult to study.

Coeliac disease

Definition and epidemiology

Coeliac disease is a chronic immune-mediated inflammatory disease that affects the small intestine and is triggered by gluten exposure in a genetically susceptible host. Coeliac disease affects about 1% of the population worldwide and is associated with increased morbidity and mortality[199-202]. A meta-analysis published in 2019 revealed that the incidence of coeliac disease has been increasing by 8.4% (95% CI 6.0–10.8%) annually over the past few decades (since the 1990s), with female predominance[203]. Coeliac disease can occur at any age, and studies have demonstrated that a loss of gluten tolerance can occur during adulthood[204]. Although increased incidence and prevalence might reflect improved detection, disease development in adulthood and the dramatic increases observed suggest that environmental factors are contributing to the risk of coeliac disease.

Environmental factors

Coeliac disease has been strongly linked to HLA variants within the DQ2 and DQ8 heterodimers[205]. Although the presence of risk alleles is generally necessary for coeliac disease development, it is not sufficient, indicating the importance of environmental factors[205]. Earlier studies evaluating the microbial populations associated with coeliac disease found intestinal dysbiosis in patients, with an increased abundance of *Escherichia coli* and *Bacteroides* and a decreased abundance of *Bifidobacterium*[206-211]. Some differences in relative abundance resolve with a gluten-free diet, whereas others persist[206-211]. In 2015, Galipeau et al. showed that in mice with genetic susceptibility (that is, expressing the human HLA-DQ8 gene) for coeliac disease, antibiotic exposure influenced gluten-induced immunopathogenicity that depended on the specific bacterial taxa expanded by the antibiotic[212]. These findings are consistent with cohort studies showing that an increased risk of coeliac disease is associated with such microbiome perturbations as caesarean delivery and proton pump inhibitor exposure[213,214], but studies now suggest that particular taxa are potentially implicated in pathogenesis.

Additional evidence is emerging that gut microbiota composition and function contribute to the development of coeliac disease in genetically susceptible hosts[212,215-217]. Opportunistic pathogens can induce immune activation of gluten-specific T cells relevant for coeliac disease, either through bacterial elastase modification (increasing immunogenicity and mucosal translocation)[215,216] or via molecular mimicry[217] in animal and preclinical studies. Caminero et al. colonized germ-free mice with opportunistic pathogens derived from small intestinal biopsy samples from patients with coeliac disease, including *Pseudomonas aeruginosa,* or with *Lactobacillus* spp. from healthy control individuals[216]. *P. aeruginosa* showed enhanced mucosal translocation in the mouse intestine[216], and *P. aeruginosa*-modified gluten peptides which were then recognized by activated gluten-specific T cells from patients with coeliac disease, leading to increased immune recognition. By contrast, *Lactobacillus* spp. from healthy individuals degraded gluten peptides, resulting in decreased immunogenicity[216]. Taken together, multiple factors modulated by specific opportunistic pathogens associated with coeliac disease could reduce tolerance towards gluten in genetically susceptible individuals.

Clinical studies

Coeliac disease and antibiotic exposure.—To date, there have been ten studies evaluating antibiotic exposure and risk of developing coeliac disease (TABLE 3). In addition, systematic reviews and meta-analyses of the literature from 2018 to 2020 have demonstrated increased risk of coeliac disease and antibiotic exposure[218-220]. Jiang et al. found increased risk of coeliac disease after antibiotic exposure (pooled OR 1.2, 95% CI 1.04–1.39), and specifically for antibiotic exposure during childhood (OR 1.15, 95% CI 1.02–1.29)[218]. Kamphorst et al. also concluded that antibiotic exposure in the first 2 years of life is associated with coeliac disease risk[219]. Although the studies evaluated in this meta-analysis were of high quality, there were only four, and the odds ratios were modest, ranging from 1.13 to 1.4 (REFS.[221-223]). However, the clear dose–response relationship in three of the studies provides further evidence of a relationship[221-223] (TABLE 3). In another population-based birth cohort study of >14,000 children born in Olmsted County, Minnesota, between January 2003 and December 2011, antibiotic exposure in the first 2 years of life was studied as a possible risk factor for the development of ten conditions with childhood onset, including coeliac disease. In the cohort, which included 45 children who were subsequently diagnosed with coeliac disease, there was a significant antibiotic dose-dependent relationship, which was stronger in girls than in boys.

Timing of antibiotic exposure.—Five studies showed an increased risk of coeliac disease associated with antibiotic exposure. In four of the studies, the exposure was within the first 2 years of life[94,221-223], whereas the fifth study examined exposures at all ages in childhood[224] (TABLE 3). It is biologically plausible to interpret these findings as supporting a causal relationship given that the gut microbiota becomes well-established by 3 years of age[6]. Both studies that evaluated maternal (prenatal) antibiotic exposure did not show a statistically significant difference in the risk of coeliac disease in the offspring between prenatal exposure to antibiotics and no exposure[225,226] (TABLE 3). Taken together, these findings suggest that timing of exposure might be a significant factor for the risk of developing coeliac disease[94,221-223,225,226]. In addition, two large

cohort studies evaluated the relationships between coeliac disease and antibiotic exposure in specific at-risk populations, specifically children with T1DM with coeliac disease-permissive HLA alleles[227,228] (TABLE 3). These studies followed children from birth, based on parental reporting of antibiotic exposure, monitoring for development of positive coeliac serologies, including tissue transglutaminase IgA, and in one study for development of histologically proven coeliac disease[227]. In these restricted populations, neither study showed an association between antibiotic exposure and the development of either coeliac disease or positive serologies.

Pathogenesis

Several high-quality studies have shown associations between the development of coeliac disease and early-life antibiotic exposure with dose-dependent relationships[94,221-223]; however, we know little about the specific mechanisms, other than the evidence of major changes in immunological development seen in other studies[10,68,94,166]. There seems to be a critical window, perhaps within the first 2 years of life, in which antibiotic exposure, by perturbing the gut microbiome and consequently altering immunological maturation, affects coeliac disease development. However, other windows might exist in later life, given that coeliac disease can develop in adulthood. Certain HLA haplotypes affect disease onset. For example, having two copies of *HLADQB1*02* has been associated with earlier disease onset, classic clinical presentation and more severe histological damage[229]. Future studies to help better understand the interplay between genetic susceptibility and environmental contributions, such as that from a perturbed microbiota, should also include HLA genotyping. Our understanding of the pathogenetic steps has been limited by the lack of a proper animal model. The development of a mouse model in 2020 that approximates coeliac disease through overexpression of IL-15 and expression of the predisposing HLA-DQ8 molecule, leading to development of villous atrophy after ingestion of gluten, has great promise for developing a greater mechanistic understanding[230].

Discussion and conclusions

In this Review, we consider several distinct diseases. Yet, all are centred on the development of abnormal patterns of inflammation of the gastrointestinal tract, an organ system that hosts an enormous, complex and varied microbiota. Although our discussion of the aetiology of these diseases centres on perturbation of the hindgut microbiome, we also consider another disease that is increasing in incidence, EoE (TABLE 4), for which foregut microbiota perturbation might be important (BOX 1). In reality, the principles being considered are parallel for the foregut and hindgut.

For each of these diseases, there is a growing body of evidence that a perturbed microbiota is associated with onset and pathogenesis. There is a complex interplay between host genetics and environmental factors that influence gut microbiota composition and functionality, with multiple confounding factors. Epidemiological studies suggest that antibiotic treatments, especially in early life, are associated with increased risk of these diseases by altering the microbiota. However, as they are designed to test associations rather than causal roles, they can never be conclusive. However, experimental studies of several antibiotic

treatments in mice have shown substantial early-life perturbations of the gut microbiota, with downstream metabolic and immunological effects. From such experiments, it is possible to reach conclusions about causality (in mice), which can be juxtaposed with the human epidemiological and clinical data to reach broader conclusions. Experiments further indicate that variation in antibiotic types, dosages and timing could explain differences in disease risk.

If this notion is correct, then it can help us to understand the pathogenesis of each of these diseases in new ways, beginning with how microbial populations in the lumen signal to host tissues in beneficial or pathogenic ways. Understanding the pathogenetic mechanisms might lead to new approaches to the diagnosis and treatment of these diseases. In addition, such investigations should lead us to a new appreciation of the biological costs of antibiotic treatments. Although antibiotic treatment can be life-saving, most patients receive antibiotics for treatment of mild infections, often without strong indications of utility[22]. Such wide use reflects a general sense by both practitioners and the public that antibiotics are very safe, and that benefits outweigh any risks. But if antibiotic exposure is indeed playing a part in any or all of these diseases, then consideration of their risks must grow, and their use must be tempered by a more transparent risk–benefit assessment.

Antibiotics have been pillars of medicine for the past 75 years, and have so much benefit and so little immediate cost that they are widely used even for the most marginal indications[22]. However, increasing evidence of long-term costs propels us to find alternative approaches to control bacterial infections. One important avenue is the development of narrow-spectrum agents, whether they be antibiotics, peptides, bacteriophages or other approaches, to reduce the unintended collateral consequences of broad-spectrum agents. Other approaches are ecological: to select for, or introduce, competitors of the pathogens; this could be done with single agents or mixtures of beneficial organisms, using probiotics, prebiotics or FMT. Retreating from antibiotic 'carpet bombing' of the intestinal microbiota will probably prevent much future disease.

However, as discussed throughout this Review, the data are incomplete, and limited by the complexity of testing hypotheses that involve exposures months, years or even decades before the development of a disease. Going forwards, these issues should be considered in light of the nine criteria for understanding causal relationships developed in 1965 by Austin Hill[231]. These criteria, which include the strength of the association (effect size), consistency (reproducibility), specificity, temporality, biological gradient (dose–response relationship), plausibility, coherence, experiment and analogy, are highly relevant to the questions raised here. For each of the illnesses, there is a partial match with the criteria, but the data are incomplete to fully assess the link and its magnitude. As such, data interpretation in humans is challenging, and many pathways remain to be discovered (BOX 2). Further knowledge will require more prospective epidemiological studies, clinical trials, and disease models in experimental animals that mimic the conditions of interest. Nevertheless, this interface involving some of the most common medicines used in the world represents an important frontier of medical science for diseases of global significance.

Acknowledgements

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Key points

- The widespread use of antibiotics worldwide is consistent with a rise in chronic inflammatory diseases of the gastrointestinal tract, including inflammatory bowel disease, coeliac disease, eosinophilic oesophagitis, and type 1 and type 2 diabetes.

- Exposure to antibiotics leads to profound effects on both the composition and the functionality of the gut microbiota, leading to potential pathogenic mechanisms for disease onset.

- Experimental studies have shown that antibiotic-induced perturbations of the microbiota are transferable and affect disease development.

- Differential levels of antibiotic exposures, and their types and timing — particularly exposure in early childhood — could explain differences in disease risk.

- A growing body of evidence indicates that an antibiotic-perturbed microbiota is associated with disease development, although current knowledge is limited by microbiota complexity. Research including prospective epidemiological studies, clinical trials and experimental studies is required.

- Novel therapies aiming to remediate the perturbation of the gut microbiome are being researched, including prebiotics, probiotics, synbiotics and faecal microbiota transplantation; however, the strong application of antibiotic stewardship is most warranted to prevent perturbing the microbiome.

Lipopolysaccharide (LPS).

A microbiota-derived endotoxin found in the outer membranes of Gram-negative bacteria. Bacterial LPS has a key role as an elicitor of innate immune responses through binding to CD44, LBP and Toll-like receptor 4.

Germ-free mice

Mice born and raised in sterile conditions and thus free of bacteria and fungi.

Dysbiosis

Perturbation of the homeostasis of gut microbiota composition, potentially leading to changes in both functional and metabolic activities.

Short-chain fatty acids (SCFAs).

Predominantly butyrate, propionate and acetate. Metabolic products of complex carbohydrate fermentation, chiefly from anaerobic bacteria, that are both energy sources for hosts as well as signalling molecules to host tissues.

Probiotics

Viable microorganisms that reach the intestine in an active state and might elicit a favourable effect on host metabolism or re-establishment of gut microbial composition.

Non-obese diabetic mice (NOD mice).

A mouse strain developing an autoimmune illness resembling type 1 diabetes mellitus in humans. The substrain NOD/Caj have membrane-bound immunoglobulins and do not secrete antibodies, so as to understand the role of B cells as antigen-presenting cells rather than production of auto-antibodies only.

Prebiotic

Dietary components, mostly consisting of non-digestible fibres, that might have a potential beneficial effect on gut microbial composition and function.

Synbiotic

Nutritional supplements that consist of a synergistic combination of both prebiotics and probiotics.

Box 1 |

Eosinophilic oesophagitis

Eosinophilic oesophagitis (EoE) is a chronic immune-mediated disease that is defined by the presence of symptoms consistent with oesophageal dysfunction, with an oesophageal biopsy sample showing 15 eosinophils per high power field, while excluding other causes of oesophageal eosinophilia[244,245].

The immune response in EoE is mainly mediated by T helper 2 (T_H2) interleukins[244,246]; overexpression of IL-13 selectively induces eotaxin 3 (also known as CCL26) expression in oesophageal epithelial cells, leading to eosinophilic infiltration and activation within oesophageal tissue[246-249].

Epidemiology

As with other atopic diseases, the prevalence of EoE has increased in the past few decades[250-254]. EoE now accounts for approximately one-quarter of histologically proven oesophageal disease in children undergoing oesophagogastroduodenoscopy (EGD)[250,251]. EoE affects all age groups, is male-predominant, and is global, although it seems to be more common in temperate climates and in those of European descent[244]. Improved detection alone does not account for the increase in prevalence[250,252,253,255,256]. Although genetic variants might relate to EoE risk, a retrospective cross-sectional study utilizing two cohorts, including an international registry of EoE twin probands, showed that there was a stronger environmental contribution than genetic contribution to EoE[255]. In the twin cohort, genetic heritability was $14.5 \pm 4\%$, and the common family environment contributed $81 \pm 4\%$ to risk[255].

Inverse association with *Helicobacter pylori*

Helicobacter pylori has been inversely associated with atopic diseases such as asthma[257] and oesophageal diseases including gastroesophageal reflux disease (GERD)[258-261], Barrett oesophagus[261] and oesophageal adenocarcinoma[262]. Six studies have shown an inverse relationship between the presence of *H. pylori* and either oesophageal eosinophilia and/or EoE[263-268]. Humans have been colonized by *H. pylori* for at least 100,000 years, and probably longer[118]. Loss of key microbial species, including *H. pylori,* as a result of antibiotic exposures could plausibly contribute to EoE risk[269].

Clinical studies

In six clinical studies evaluating the relationship between antibiotic exposure and EoE risk, the odds ratios ranged from 1.3 to 6 (REFS.[233-238]) (TABLE 4). A 2021 meta-analysis of five of these studies showed significant associations between antibiotic exposure and EoE risk in four studies[219,233-236]. In one study, there were similar rates of early-life exposure to antibiotics between EoE and GERD cohorts (81% and 73%, respectively), which were higher than the exposure rate (42%) for a control cohort of asymptomatic children[234]. Although these findings provide evidence that antibiotic exposure in early life might increase the risk of developing EoE, they are limited by the small numbers of individuals studied.

Timing of EoE onset and antibiotic exposure.

A case–control study evaluated patients with EoE who did not develop symptoms until 18 years of age, nested within a prospective cohort study of adults undergoing EGD for evaluation of gastrointestinal symptoms[237]. Both the individuals and their mothers provided information about early-life antibiotic exposures; antibiotic exposure in the first year of life was associated with adult-onset EoE (OR 4.6)[237] (TABLE 4).

EoE disease severity and antibiotic exposure.

In a retrospective review of Italian children and adults with EoE, antibiotic exposure in relation to EoE disease activity was studied[270]. The researchers defined refractory EoE as symptom 'flare-ups' and responsive EoE as asymptomatic, but histological data to confirm EoE disease activity was not reported. Antibiotic exposure was defined as repeated courses (three or more courses per year) in the first 3 years of life. Adults with refractory (symptomatic) EoE were significantly more likely to have repeated antibiotic exposure (as defined by the authors): 70% versus 33.3% in asymptomatic EoE ($P = 0.03$)[270]. Further studies are needed to assess the relationship between antibiotic exposure and EoE disease severity.

Box 2 |

Open research questions

- What is the relationship of the timing of antibiotic exposure to disease risk?

- Do all antibiotics have similar metabolic and immunological effects mediated by microbiota disturbances?

- Are there particularly bad combinations of antibiotic exposures that magnify risk?

- What is the relationship between antibiotics and other exposures that perturb the early-life microbiome (for example, caesarean birth, formula feeding)?

- What are the mechanisms by which a perturbed microbiota aberrantly signals to host tissues?

- After a damaging exposure, can there by restoration to baseline?

  - What is the relevant time window?

  - Is there a point of no return?

  - What are the optimal ways to accomplish this (for example, prebiotic, probiotic or synbiotic supplementation, or faecal microbiota transplantation)?
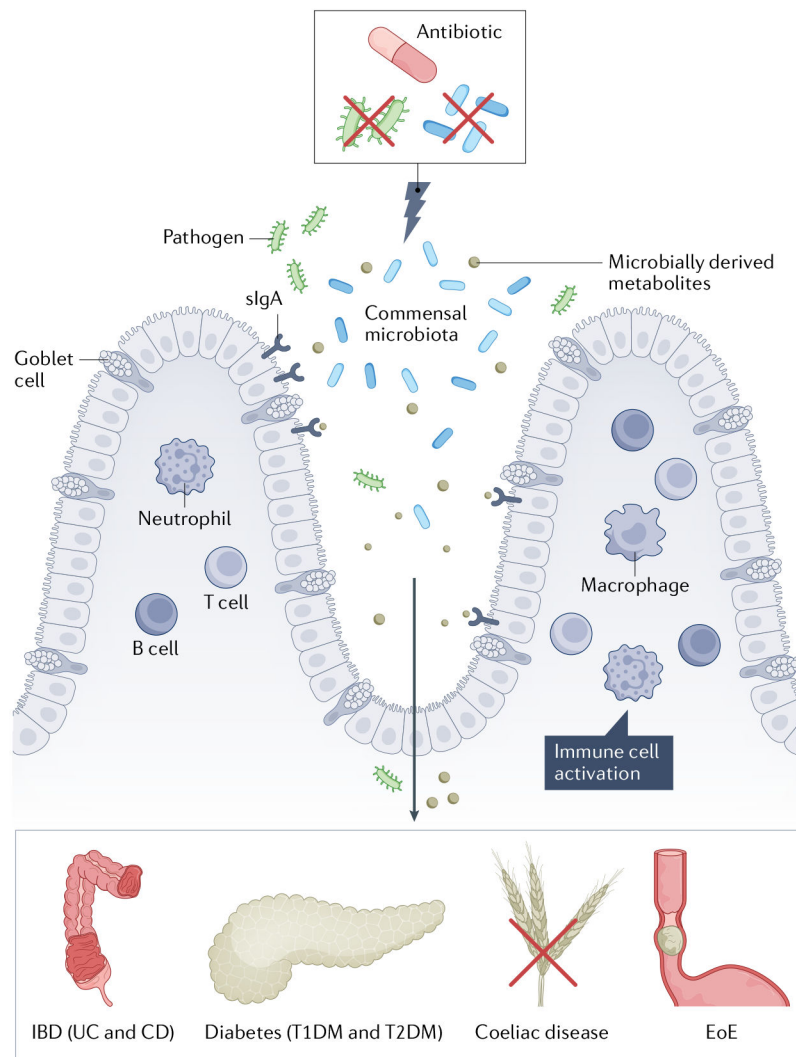
Fig. 1 |. The gut microbiota and antibiotics in the pathogenesis of inflammatory diseases of the gastrointestinal tract.
Schematic overview of the role of the microbiota in the pathogenesis of inflammatory diseases injuring organs in the gastrointestinal tract, leading to the onset and development of both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), inflammatory bowel disease (IBD; including ulcerative colitis (UC) and Crohn's disease (CD)), coeliac disease and eosinophilic oesophagitis (EoE). Exposure to antibiotics leads to profound effects on both the composition and functionality of the gut microbiome, leading to decreased diversity. These changes might then lead to secondary effects involving the intestinal wall including altered epithelial cell signalling to adaptive immune effectors, and/or increased intestinal permeability, leading to translocation of microbial constituents and products into the systemic circulation, among other mechanisms. sIgA, secretory IgA.
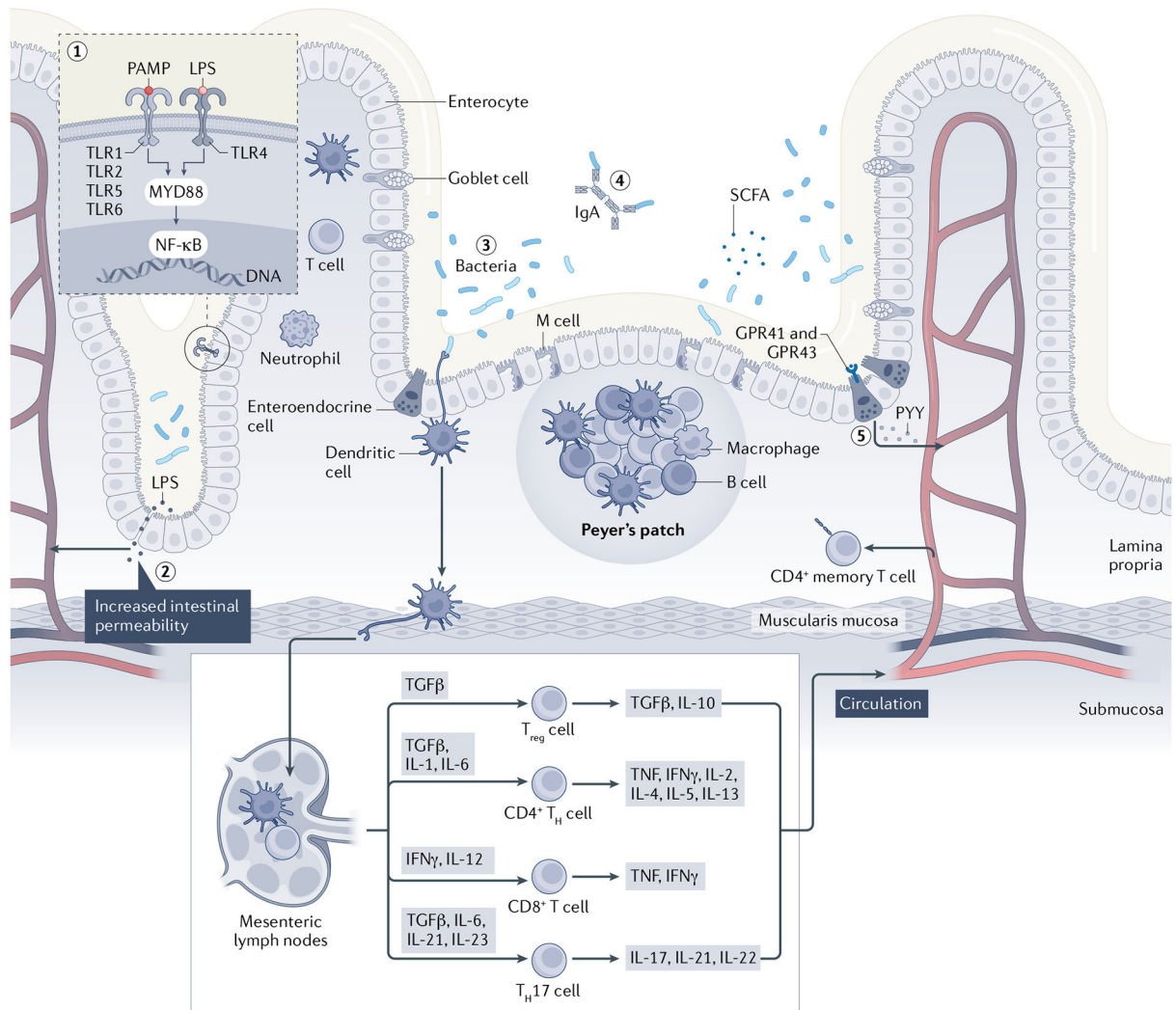
Fig. 2 |. Complex interplay between the gut microbiota and the immune system in diabetes and IBD.

Microbially derived peptides, such as pathogen-associated molecular patterns (PAMPs) and lipopolysaccharide (LPS), bind to Toll-like receptors (TLRs) on the cell membrane of enterocytes (1). Activation of these TLR/MYD88-dependent signalling pathways leads to translocation of nuclear factor-κB (NF-κB) into the nucleus, promoting transcription of numerous cytokines[63,65,239]. Dysbiosis of the gut microbiota can lead to intestinal barrier dysfunction and increased intestinal permeability (2). This facilitates the translocation of PAMPs and LPS into the systemic circulation, leading to a persistent, low-grade inflammatory state of liver, muscles, and visceral and subcutaneous adipose tissue as observed in both diabetes (both type 1 and type 2 diabetes mellitus) and inflammatory bowel disease (IBD)[109-114]. Across the intestinal epithelium, antigen-presenting cells (APCs), including macrophages and dendritic cells, detect pathogenic bacteria and promote the antigens on the cell surface (3). Thereafter, the APCs migrate to mesenteric lymph nodes, mediating an alteration of T lymphocyte subsets[66,71]. Secretory IgA (sIgA) serves as the first line of defence in protecting the intestinal epithelium from enteric toxins and pathogenic

microorganisms (4). Antibiotic exposure leads to lower levels of sIgA, potentially leading to an increased inflammatory environment[9]. Gut microbiota ferment diet-derived carbohydrates into short-chain fatty acids (SCFAs) (5). SCFAs are ligands of the G protein-coupled receptors GPR41 and GPR43, which are expressed by intestinal enteroendocrine cells and enhance production of peptide YY (PYY), a hormone that affects insulin utilization by increasing the intestinal transit time, and increases satiety and energy harvest from the diet[105,106]. T_H cell, T helper cell; T_{reg} cell, regulatory T cell. The original version of this figure was created with BioRender.com.
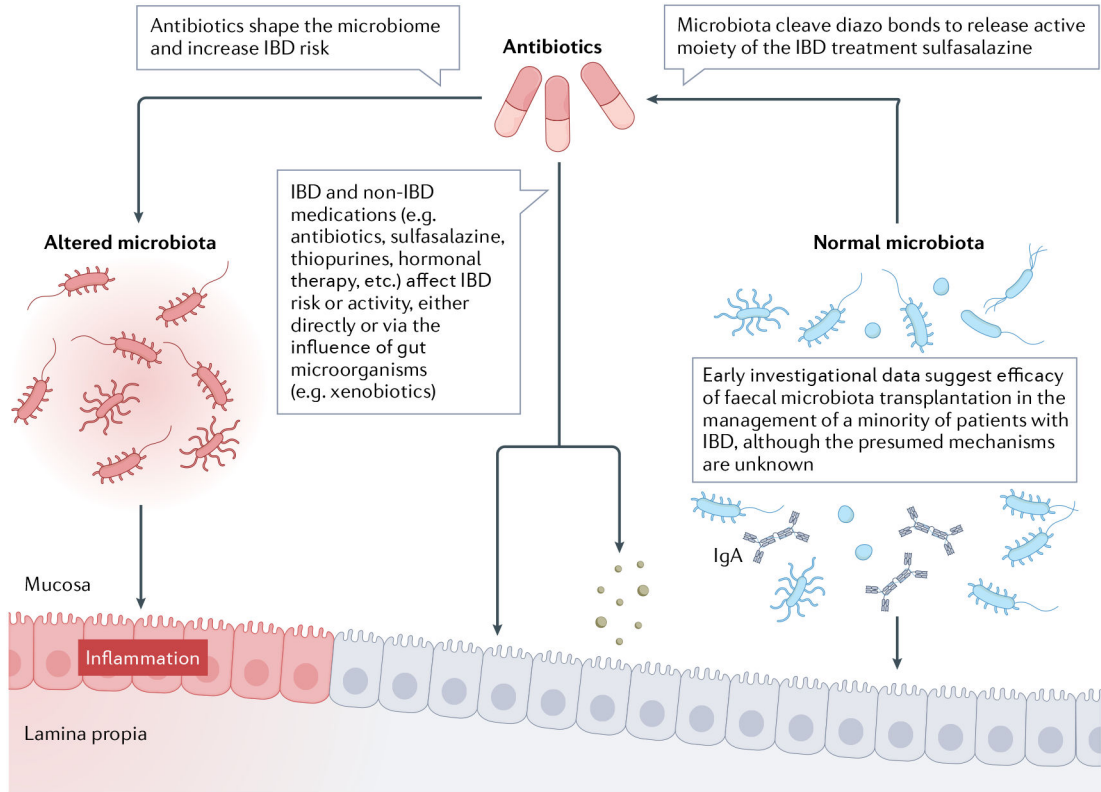
Fig. 3 |. Influence of the gut microbiota on inflammatory bowel disease activity.
Antibiotics shape the microbiota and increase the risk of inflammatory bowel disease
(IBD)[150-158,161]. Normal microbiota constituents cleave sulfa-diazo bonds to release the
active moiety of the IBD treatment sulfasalazine[186,187]. IBD and non-IBD medications
(for example, antibiotics, sulfasalazine, thiopurines, hormonal therapy, etc.) affect IBD
risk or activity, either directly or with the influence of gut microorganisms (for
example, xenobiotics)[150-158,161,175-180,183,184,186-190,240-242]. Early investigational data
suggest efficacy for crude faecal microbiota transplantation in IBD management, although
the precise mechanism is unknown[191,193,243]. The original version of this figure was created
with BioRender.com.

Table 1 |

Clinical studies evaluating antibiotic exposure and risk of diabetes and childhood obesity

Study	Antibiotic exposure timing	Date (location)	Study design	Antibiotic data source	Disease diagnosis	Key findings [a]
Type 1 diabetes mellitus						
Wernroth et al.[50]	Prenatal to 12 months	2020 (Sweden)	Cohort	Prescriber registry (ATC code)	Database diagnostic code for T1DM (ICD-10: E10)	Increased risk of T1DM before age of 10 years: 1 year: aHR 1.19 (1.05–1.36); 44.3/100,000 person-years among exposed children versus 39.0/100,000 person-years among non-exposed children; 6 months: aHR 1.26 (1.04 – 1.5) Modified by mode of delivery
Clausen et al.[51]	Birth to 24 months	2016 (Denmark)	Cohort	Prescriber registry (ATC code)	Database diagnostic code for T1DM	Increased risk after exposure to broad-spectrum antibiotics HR 1.13 (1.02–1.25) Modified by mode of delivery
Hviid and Svanström[54]	12 months to year 2005	2009 (Denmark)	Cohort	Prescriber registry (ATC code)	Database diagnostic code for T1DM (ICD-10: E10)	Differences not significant RR 1.16 (0.91–1.50)
Tapia et al.[55]	Prenatal to 18 months	2018 (Norway)	Cohort	Repeated questionnaires	Database diagnostic code for T1DM (ICD-10: E10)	Differences not significant Prenatal: aHR 1.09 (0.85–1.35) In early life: aHR 1.11 (0.81–1.50)
Childhood obesity						
Trasande et al.[90]	<6 months 6–14 months 14–23 months	2013 (UK)	Longitudinal birth cohort	Repeated questionnaires	Measured during five study visits	Increased body mass at 10–38 months, after antibiotic exposure during first 6 months of life aOR 1.22 (P = 0.029)
Bailey et al.[91]	0–23 months 24–59 months	2014 (Philadelphia, USA)	Cohort	Outpatient prescriptions and patient-reported medications	Measured during recurring study visits	Cumulative exposure to antibiotics was associated with development of obesity 4 courses: RR 1.11 (1.02–1.21) Stronger effect for broad-spectrum antibiotics: RR 1.16 (1.03–1.19)
Azad et al.[92]	0–5 years	2014 (Canada)	Longitudinal birth cohort	Prescription records	Measured at 9 and 12 years of age	Increased risk of overweight and central adiposity in preadolescent boys, but not girls Age 9 years: aOR 2.19 (1.06–4.54) Age 12 years: aOR 5.35 (1.94–14.72)
Murphy et al.[93]	0–12 months	2014 (18 countries/regions)	Cross-sectional	Repeated questionnaires	Self-reported or measured	With antibiotic exposure, increased childhood BMI in boys aged 5–8 years, but not girls: BMI + 0.107 kg/m^2 (P < 0.0001)
Aversa et al.[94]	0–6 months 6–12 months 12–24 months	2021 (Minnesota, USA)	Population-based cohort	Medical records linkage system	Medical records linkage system	Increased risk for overweight and obesity, depending on number, type and timing of antibiotic exposure Girls with overweight: HR 1.19 (1.09–1.30) Girls with obesity: HR 1.13 (0.99–1.29) Boys with overweight: HR 1.22 (1.12–1.34) Boys with obesity: HR 1.12 (1.08–1.39)

Study	Antibiotic exposure timing	Date (location)	Study design	Antibiotic data source	Disease diagnosis	Key findings[a]
Mueller et al.[48]	Prenatal	2014 (New York, USA)	Cohort	Questionnaire	Measured at age 7 years	Exposure to antibiotics in the second or third trimester associated with higher risk of childhood obesity aRR 1.77 (1.25–2.51)
Mbakwa et al.[95]	Birth to 10 years	2016 (Netherlands)	Longitudinal cohort	Repeated questionnaires	Self-reported over 7 time points	Increased height and weight in children exposed to: One course during first 6 months of life; adjusted β 0.24 and 0.23 Two or more courses during second year of life; adjusted β 0.34 and 0.29
Type 2 diabetes mellitus in adults						
Mikkelsen et al.[87]	Adulthood	2015 (Denmark)	Case–control	Prescriber registry (ATC code)	First-ever prescription of a non-insulin glucose-lowering agent (ATC A10B)	Dose-dependent relationship with number of antibiotic courses: For 2–4, OR 1.21 (1.19–1.24) For 5, OR 1.53 (1.50–1.55)
Davis et al.[88]	Adulthood	2019 (USA)	Retrospective cohort	Outpatient antibiotic prescriptions >6 months prior to diabetes diagnosis	Two or more ICD-9 codes for diabetes or two or more prescriptions of diabetes medications, other than metformin	Increased risk after exposure to more than one prescription of antibiotics HR = 1.13 (1.01–1.25)
Boursi et al.[89]	Adulthood	2015 (UK)	Nested case–control	Antibiotic prescriptions >1 year before diabetes diagnosis	At least one Read code (general practitioners)	No significant difference after a single antibiotic course Dose-dependent relationship for number of antibiotic courses (two or more), with OR depending on antibiotic type

aHR, adjusted hazard ratio; aOR, adjusted odds ratio; aRR, adjusted relative risk; ATC, Anatomical Therapeutic Chemical classification system; ICD, International Classification of Disease; RR, rate ratio; T1DM, type 1 diabetes mellitus.

[a]Value ranges in parentheses are 95% confidence intervals; adjusted β are adjusted generalized estimating equation estimates in relation to z-scores.

Table 2 |

Clinical studies evaluating antibiotic exposure and inflammatory bowel disease

Study	Antibiotic exposure timing	Date (location)	Study design	Antibiotic data source	Disease diagnosis	Key findings [a]
Kronman et al.[150]	Childhood	2012 (UK)	Retrospective cohort	Health records registry	Diagnosis (Read) codes	HR IBD 5.51 (1.66–18.28) — antibiotic exposure before 1 year of age; HR 2.62 (1.61–4.25) — by 5 years of age; HR 1.57 (1.35–1.84) — by 15 years of age Dose dependency
Hviid et al.[151]	Childhood	2011 (Denmark)	Retrospective cohort	National health registry	Diagnosis (ICD) codes	IBD RR 1.84 (1.08–3.15) Dose dependency
Nguyen et al.[152]	All	2020 (Sweden)	Case–control	National health registry	Diagnosis (ICD) codes	aOR IBD 1.94 (1.85–2.03) Dose dependency
Shaw et al.[153]	Infants <1 year of age	2010 (Manitoba, Canada)	Nested case–cohort	Provincial health registry	Diagnosis (ICD) codes	OR IBD 2.9 (1.2–7.0) Dose dependency
Shaw et al.[154]	2–5 years before diagnosis	2011 (Manitoba, Canada)	Case–control	Provincial health registry	Diagnosis (ICD) codes	aOR IBD 1.27 (1.20–1.35) Dose dependency
Card et al.[157]	2–5 years before diagnosis	2004 (UK)	Case–control	Research database	Diagnosis (Oxmis, Read) codes	aOR IBD (1.05–1.65) No dose dependency
Virta et al.[158]	Childhood	2012 (Finland)	Case–control	National health registry	Diagnosis (ICD) codes	aOR CD 1.87 (1.37–2.56) aOR UC 1.18 (0.92–1.52)
Ungaro et al.[155]	Variable	2014 (Europe, Canada, New Zealand)	Meta-analysis	Mostly health registries, some surveys	Varies	Pooled OR IBD 1.57 (1.27–1.94)
Zou et al.[156]	Childhood	2020 (North America, Eurasia, Oceania)	Meta-analysis	Varies	Varies	Pooled OR IBD 1.5 (1.22–1.85)

aOR, adjusted odds ratio; CD, Crohn's disease; IBD, inflammatory bowel disease; ICD, international Classification of Disease; RR, rate ratio; UC, ulcerative colitis.

[a] Value ranges in parentheses are 95% confidence intervals.

Table 3 |

Clinical studies evaluating antibiotic exposure and coeliac disease risk

Study	Antibiotic exposure timing	Date (location)	Study design	Antibiotic data source	Coeliac disease diagnosis	Key findings[a]
Mårild et al.[225]	Prenatal	2014 (Sweden)	Cohort	Prospective questionnaire	Histology (Marsh 3) and either positive coeliac serologies or symptoms consistent with coeliac which resolved on GFD[b]	No significant difference HR 1.33 (0.69–2.56)
Mårild et al.[226]	Prenatal	2017 (Norway)	Cohort	Prospective questionnaire	Questionnaire or database diagnostic codes for coeliac disease[c]	No significant difference aOR 1.16 (0.94–1.43)
Myléus et al.[232]	Birth to 6 months	2012 (Sweden)	Case–control	Parental questionnaire	Three consecutive duodenal biopsy samples (Marsh 3)	No significant difference between coeliac disease and controls OR 1.2 (0.87–1.6)
Canova et al.[221]	Birth to 12 months	2014 (Italy)	Cohort	Prescriber registry	Database diagnostic codes for coeliac disease	OR 1.3 (1.10–1.56) Dose–response relationship with five or more antibiotic courses OR 2.66 (1.79–3.95)
Dydensborg Sander et al.[222]	Birth to 12 months	2019 (Denmark and Norway)	Observational cohort	Prescriber registry	Database diagnostic codes for coeliac disease	OR 1.26 (1.16–1.36) Dose-dependent relationship for each additional antibiotic OR 1.08 (1.05–1.11)
Kemppainen et al.[228]	Birth to 48 months	2017 (Finland, Germany, Sweden and USA (TEDDY))	Cohort with T1DM and permissive HLA for CD	Prospective questionnaire	Risk of coeliac disease defined as two consecutive positive serum TTG IgA at least 3 months apart[d]	No increased risk of positive TTG IgA and antibiotic exposure HR 1.00 (0.98–1.02)
Bittker and Bell[223]	Birth to 48 months	2019 (USA)	Case–control Internet-based survey	Parental report	Diagnosis from medical professional	aOR 1.13 (1.04–1.24) Dose-dependent relationship for number of antibiotic courses For four to seven, OR 1.62 (1.03–2.55) For eight or more, OR 2.48 (1.29–4.75)
Aversa et al.[94]	Birth to 24 months	2021 (USA)	Cohort	Prescriber registry	Database diagnostic codes for coeliac disease	Dose-dependent relationship Gender specific for girls: For one or two antibiotic prescriptions, HR 8.12 (1.03–64.10) For more than five antibiotic prescriptions, HR 12.32 (1.56–97.32)
Simre et al.[227]	Birth to 60 months	2016 (Estonia and Finland (DIABIMMUNE))	Cohort with T1DM and permissive HLA for CD	Parental report of antibiotic exposure	Positive coeliac serology and duodenal biopsy sample (Marsh 3)	No significant difference (number of antibiotic courses 1.1 versus 1.0 Finland)
Mårild et al.[224]	All ages	2013 (Sweden)	Case–control	Prescriber registry	Histology database (Marsh 3)	OR 1.40 (1.27–1.53) Also found increased risk with inflammation (Marsh 1 and 2) and those with normal

Study	Antibiotic exposure timing	Date (location)	Study design	Antibiotic data source	Coeliac disease diagnosis	Key findings[a]
						histology (Marsh 0) but positive coeliac serologies

aOR, adjusted odds ratio; CD, coeliac disease; GFD, gluten-free diet; OR, odds ratio; T1DM, type 1 diabetes mellitus; TTG, tissue transglutaminase.

[a] Value ranges in parentheses are 95% confidence intervals.

[b] Data obtained from prior study published in 2004.

[c] Validation study performed by authors.

[d] Primary outcome was risk of coeliac disease.

Table 4 |

Clinical studies evaluating antibiotic exposure and eosinophilic oesophagitis risk

Study	Timing of antibiotic exposure	Age of EoE onset	Date (location)	Study design	Antibiotic data source	EoE diagnosis	Key findings[a]
Witmer et al.[233]	Birth to 6 months	Paediatric	2018 (USA)	Case–control	Pharmaceutical coding records	National military database	aOR 1.31 (1.10–1.56)
Jensen et al.[234]	Birth to 12 months	Paediatric	2013 (NC, USA)	Case–control	Retrospective survey	Database[b]	OR 6 (1.7–20.8)
Radano et al.[235]	Birth to 12 months	Paediatric	2014 (Boston, MA, USA)	Case–control	Retrospective questionnaire	Database[b]	OR 3.61 (1.11–11.7)
Jensen et al.[236]	Birth to 12 months	Paediatric	2018 (Cincinnati, OH, USA)	Case–control	Retrospective questionnaire	Database[b]	aOR 2.30 (1.21–4.38)
Dellon et al.[237]	Birth to 12 months	Adult	2021 (NC, USA)	Nested case–control[c]	Retrospective questionnaire	Database[b]	OR 4.64 (1.63–13.2)
Slae et al.[238]	Birth to 12 months[d]	Paediatric	2015 (Canada)	Case–control	Retrospective questionnaire	Database[b]	No significant difference OR 1.00

aOR, adjusted odds ratio; EoE, eosinophilic oesophagitis; OR, odds ratio.

[a]Value ranges in parentheses are 95% confidence intervals.

[b]Database included histopathological data to confirm EoE diagnosis based on accepted definitions.

[c]Case–control study nested within a previously conducted prospective cohort study of adults undergoing outpatient oesophagogastroduodenoscopy for evaluation of gastrointestinal symptoms.

[d]This study included questions regarding recent antibiotic exposure, which was defined as 'less than once a year', 'once a year', 'two to three times per year' and 'four or more times per year'.