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PERSPECTIVE

Acute Lymphoblastic Leukemia

Can we prevent childhood Leukaemia?

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Why prevention?

Despite advances in treatment efficacy, childhood cancers continue to exert a heavy toll in morbidity and mortality. Exploring new therapeutic options has been restrained by the relative rarity of these cancers coupled with their subgroup diversity and some reluctance to invest in drug development for rare cancers.

In adult cancers, later stage or metastatic disease remains largely intransigent with emergent drug resistance as the portal for malignant escape. Whilst novel combinatorial strategies involving immunotherapy, evolutionary or adaptive control might well thwart resistance [1–3], much emphasis is placed on early diagnosis and intervention where prospects for eradication or cure are more tangible.

But it has also been argued that since the belligerence of cancer is the result of a progressive evolutionary process with highly variable dynamics Plan A for cancer control should be prevention [4]. Or, to stop it before it gets started. In theory, this makes sense but for this to be plausible, let alone practicable, requires that we can identify critical components of the causal pathway that are amenable to interception. For many common adult cancers, including breast, prostate and colorectal this remains challenging. However, the consistent, causal links between smoking and lung cancer, skin cancer and UVB and cervical cancer and HPV [5] provide hugely encouraging examples of reduction in disease burden via education, prudent avoidance and, in the case of HPV, prophylactic vaccination. There is little doubt that cancer prevention is possible and can have a substantial, global impact on public health.

For paediatric cancers including both solid tumours and leukaemia, the picture has been different. Identifying causal pathways is extremely difficult for cancers that are both rare in prevalence and biologically diverse. Moreover, the common view that many if not most childhood cancers arise via stochastic, developmental errors compounded by inherited susceptibility [6] further dampens any enthusiasm to consider prevention as a possibility.

There is however one exception to this generally pessimistic perspective and that is with childhood acute lymphoblastic leukaemia (ALL). This is the most common type of paediatric cancer (around one-third of all cases) but is itself heterogeneous, originating from multi-lineage or lymphoid progenitors. Discriminating between these subtypes has been a key component of unravelling likely causal pathways. And for the most frequent subtype, B cell precursor ALL (~75% of total), a combination of basic biological investigations and large collaborative case/control epidemiological studies has delivered a plausible causal mechanism which illuminates prospects for prevention [7].

But first, a caveat. ALL has provided one of the real success stories in oncology. Universally lethal in the absence of effective treatment [8], this cancer has been transformed by stepwise, incremental gains via systematic clinical trials of combination chemotherapy with a current cure rate of around 90% [9]. So, why should we be interested in prevention? One glib sounding but the valid answer would be to say, ‘ask any parent of a patient’. The reality is that the treatment is traumatic for very young patients and their families, and toxic with some cost or deleterious trade off in terms of morbidity and long-term health impacts [10, 11]. The excellent prospects for curative treatment are massively important to the affected families but prevention, if possible, would surely be even better?

Dissecting multifactorial causal mechanisms: where is the leverage?

Infection has, for almost a century, been considered a possible causal agent for childhood ALL [12]. But unlike leukaemias in cats, chickens and cattle [13], no specific

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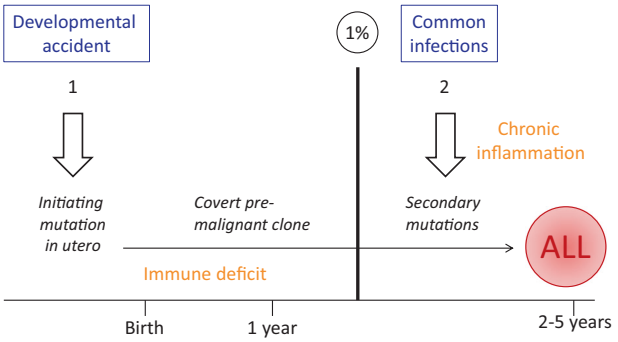

Fig. 1 The two-hit model for B cell precursor ALL. Initiating genetic lesions are primarily ETV6-RUNX1 or hyperdiploidy, probably occurring as developmental accidents. They arise in utero possibly in foetal liver early B lineage lymphopoiesis [78]. Secondary mutations are primarily RAG-mediated copy number alterations. ~1% figure: ALL is initiated in utero at a rate that exceeds by 100-fold, the incidence of disease indicating a low penetrance and a critical role for factors promoting chronic inflammation and the secondary mutations. Adapted from [7]. See text for references.

transforming virus has been identified. Instead, the current model embodies a paradox, namely that although common infections may trigger or promote this cancer the key risk factor is actually a deficit of microbial exposure, in infancy and especially in more developed or affluent societies [7, 14]. This causal model of ALL is grounded in the evolutionary, natural history of the disease, considerations of how the immune system has evolved to respond to microbial exposures and extensive epidemiological assessment of risk variables that are surrogates for common microbial exposures. The detailed evidence has been summarised recently [7]. Figure 1 presents a pictorial version of the model.

Much of the historical approach to understanding why we get cancer has courted the implicit concept of singularity of cause. This makes no more sense than singularity of cure or a magic bullet. Most if not all cancers are likely to have a multifactorial causation involving exogenous or endogenous exposures, background genetics and chance, which sit alongside evolutionary contingencies or liabilities underpinning vulnerability [4]. And ALL is no exception. For a child to develop ALL the following factors may have to come into play, collectively.

(1) The acquisition, in utero, of an initiating mutation, most commonly chromosomal hyperdiploidy or *ETV6-RUNX1* gene fusion [7]. The founder event is far more common (~100 times) than overt ALL [15, 16] and generates a persistent, covert and non-pathological pre leukaemic clone that can persist for up to at least 14 years [17]. The cause(s) of the initiating mutations is unknown but is suggested to be endogenous oxidative stress [7, 18].

(2) A small fraction (~1%) of pre leukaemias initiated in utero progress to clinical ALL, usually between the ages of two and six, after they acquire additional mutations. The latter most commonly being recombinase enzyme (RAG 1, 2) driven copy number losses of genes involved in B lineage differentiation or cell cycle control [19, 20]. The model posits that these necessary secondary mutations are an indirect consequence of a dysregulated immune response or chronic inflammation consequent to common infections [7]. There is some mechanistic insight into how this might happen [21, 22]. The infections involved are not identified, though respiratory viruses have been implicated [23, 24]. Nursery groups and schools are a likely venue or hot spot for these infections. When all schools in Hong Kong were closed for a year due to the SARS pandemic in 2003 rates of ALL declined (but not brain tumour) as did notifiable common infections in children [25]. Widespread social restrictions during the 2020 COVID-19 pandemic might be expected to have a similar impact and is currently being assessed [26].

(3) The abnormal immune response to infection in children that triggers progression to overt, clinical ALL is considered to be contingent upon a lack of microbial exposure in the first year of life which is required to prime the naïve immune network for well-regulated or balanced responses [7]. This scenario was first predicted based on immunological principles but then assessed and endorsed by case/control epidemiological studies and meta-analyses of the accumulated data [7, 27–29].

Risk is further modified by a number of inherited alleles expressed in blood cells [7, 30, 31] and may impact primarily by interacting epistatically with the endogenous mutations to drive transformation [7]. Dietary factors may also modify risk [32]. All of these variables are imbued with an element of chance and compound to provide a risk for ALL of around one in 2000 for the first 15 years of life.

The microbiome link

Of this list of risk variables, only one would seem to be potentially modifiable. This is the apparent deficit of microbial exposure in infancy. It has been unclear what these microbial infections might be and there is no association between documented pathological infections in infancy and risk of ALL. The surrogate, epidemiological variables for this exposure [7] have been day-care attendance (protective), protracted breastfeeding (protective), C section birth (increased risk) and in some but not all studies,

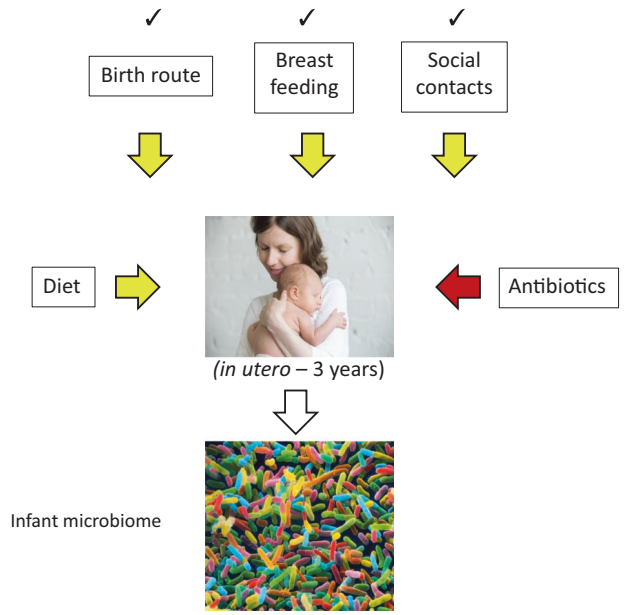

Fig. 2 Environmental, life exposures that source and impact on the infant microbiome. Of the five critical factors, three (✓ in figure) have been implicated as risk factors in B cell precursor ALL. Birth route—vaginal versus caesarean. Note: diet and antibiotics also impact on the composition of the microbiome but those two variables have not been systematically evaluated for impact on the risk of ALL.

birth order (higher risk for first borne). These variables each reflect a route via which babies and infants acquire their gut microbiomes (Fig. 2) [33–35], suggesting that the key deficit or risk factor for ALL in early life may reside in the acquisition and composition of the commensal gut microbes.

There is now substantial evidence that establishment of the gut microbiome at birth and over the first few years of life [34, 35] has profound and long-lasting effects on both metabolism and immune system function [36, 37]. The mechanisms involved are still being investigated but involve both metabolic products of bacteria [38] and direct microbial binding to Toll receptors on innate immune cells [39]. The key downstream consequence appears to be the activation of regulatory T cells that orchestrate the dynamics of immune responses [40, 41]. And the crucial, long-lasting impact is that the immune system's complex network is 'primed' or hardwired for balanced responses and avoidance of chronic inflammation.

ALL and other childhood diseases of 'affluence'

Microbial dependency of the immune system, registered very early in life, is likely to be evolutionarily ancient [42] but several aspects of modern life styles in westernised countries, including childbirth and breastfeeding practices, antibiotic use, diet and social contacts have disrupted this arrangement resulting in less diverse microbiomes or dysbiosis [43, 44]. This evolutionary mismatch might be expected to have many important pathological consequences in both children and adults for both metabolism and immune function, one of which now seems likely to be childhood leukaemia. And for children with an immune priming deficit the risks or consequences are not just for ALL.

Table 1 Shared risk factors between ALL, type 1 diabetes and allergies in children.

Risk factor for ALL	Risk	Type 1 diabetes (ref.)	Allergy (ref.)
Day-care attendance	Down	+[72][a]	+[73]
Breastfeeding	Down	+[74][a]	+[75][a]
C-section birth	Up	+[76][a]	+[77]

Risk factors for ALL (reviewed in [7]) also reported (+) for type I diabetes or allergies. There are some caveats to this summary. There is some heterogeneity of results reported and variation in parameters measured that could be important for immune priming in infancy, e.g. age and time spent in day care and length of time breastfeeding. Type of allergies or asthma measured is another variable. These data merit further scrutiny.

[a]Meta-analysis study.

Childhood allergies and type 1 diabetes are both linked epidemiologically with a deficiency of early life microbial exposure [45, 46] and more recent studies have provided some direct evidence for dysbiosis of the gut microbiome in these conditions [47, 48]. The aetiological model for these childhood diseases was originally named the 'hygiene hypothesis' but it has become clear that risk is less to do with hygiene as 'cleanliness' and more to do with 'mixed blessing' lifestyle changes that diminish opportunities for exposure to both deleterious pathogens and beneficial commensals as 'old friends' [49, 50]. Microbiome dysbiosis can therefore be considered as an unintended and deeply paradoxical consequence of 'progress'.

Childhood allergies and type 1 diabetes share many of the same early life risk factors as ALL mirroring routes of gut microbiome acquisition (Table 1). Incidence rates of all three childhood illnesses have increased over recent decades in developed societies and internationally track together with markers of affluence. Scandinavian countries topping the list [7, 45].

Could childhood ALL, allergies and type 1 diabetes, and possibly some other autoimmune diseases, such as multiple sclerosis (MS) [51], all share the same underlying, predisposing condition—an early life gut microbiome dysbiosis resulting in an immune priming deficiency? This concept might appear to be contradicted by the observation that they tend not to co-occur in families and have very distinct pathologies. But this could be explained by the impact of differing inherited susceptibility alleles plus distinctive

triggering factors targeting separate tissues. The notion that all three childhood illnesses might share the same underlying fault could, if correct, have major implications, not least for therapeutic or preventative intervention.

Prospects for microbiome boosting

Gut microbiome dysbiosis has also been linked in recent years to a number of common adult conditions including inflammatory bowel disease (IBD) and obesity [52]. Dysbiosis could also be involved in the considerable fraction of adult cancers associated with chronic inflammation [53, 54] with important implications for unpicking causation and treatment or prevention.

Notwithstanding the need for more exploration of the considerable complexities of the gut microbiome ecosystem, there is already clinical exploration of the potential clinical benefits of gut microbiome reconstitution or boosting. Examples include adult IBD [55], compensation for the microbiome deficit of C-section birth [56] and to combat the emergence of antibiotic resistant bacteria [57]. Gut microbiome reconstitution has been used for leukaemia patients having received bone marrow transplants coupled with microbiome crippling antibiotics [58]. Boosting of the gut microbiome may enhance the efficacy of immunotherapy in cancer [59].

Some of these clinical trials, and animal modelling, involve transfer of total stool samples or faecal microbiota transplants. This tactic may capture the microbial diversity of the gut microbiome, but standardised use and regulatory approval will require well defined, and non-pathogenic bacterial species. In this respect, it is encouraging that 'keystone' [60] bacterial species of the healthy infant microbiome ecosystem—*Bifidobacteria* sp., as well as *Lactobacilli*, administered with or without milk oligosaccharides, as synbiotic regimes, have provided clinical benefit or risk reduction to infants in the context of sepsis [61], allergies [62] and pre-term birth consequences [56, 63].

Collectively, these data raise the possibility that gut microbiome boosting might present a viable strategy for risk reduction or prevention in childhood ALL. A similar argument has been made for prophylactic intervention for type 1 diabetes in children [64]. But for this to be translated into practice requires additional questions to be addressed.

The challenges ahead

First, there is a need for more direct evidence that the microbiome in patients developing ALL is indeed deficient or lacking in diversity. Prospective monitoring of a very large (tens of thousands) cohort of infants might provide that evidence and such studies are initiated or in planning phases to screen for multiple heath impacts. One study [65] reports that at diagnosis, patients with ALL do have a less diverse oral microbiome than controls. This study requires scale up, confirmation for the gut microbiome and also needs to accommodate the potentially confounding effects of prior antibiotic use or the disease process itself. A proof of principle demonstration that microbiome boosting can indeed prevent infection promoted ALL in an animal model that faithfully mimics the clinical disease in children would also be very encouraging. These models are currently under development [66] (MG, VC and AF unpublished). In this respect studies on rodent models of type 1 diabetes are more advanced than leukaemia with accumulating evidence for risk reduction via microbiome-based immune modulation [45, 67–71].

But even if these issues were resolved there are several other impediments to translation of this idea into a public health measure. First, there is the substantive issue of defining the precise bacterial mix or cocktail that might be effective, coupled with the challenge of delivery and safety. But, with common adult diseases primarily in mind this is now high on the agenda in both biotech industry and academia and is likely to be resolved soon. Second, there is the question of who would receive any potential protective treatment in infancy. Although the main risk factors for ALL are now recognised, it remains very difficult to identify individuals at risk in the population. Any prophylactic intervention might therefore have to be unselective or population wide. How would this be justified for a cancer that is rare and largely curable? It could be argued that the current suite of vaccines given to young children provide a precedent but this might prove unpersuasive.

There is one strategy that could be taken to both address this challenge and maximise potential benefit. This is to ask the audacious question of whether population wide microbiome boosting in infancy, with a single defined, and safe, bacterial preparation, might not deliver multiple health benefits, including risk reduction for childhood leukaemia, allergies and autoimmune disease. And, very likely, long-term benefits for adult health.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

1. Al-Lazikani B, Banerji U, Workman P. Combinatorial drug therapy for cancer in the post-genomic era. Nat Biotechnol. 2012;30:679–92.
2. Gatenby RA, Silva AS, Gillies RJ, Frieden BR. Adaptive therapy. Cancer Res. 2009;69:4894–903.
3. Acar A, Nichol D, Fernandez-Mateos J, Cresswell GD, Barozzi I, Hong SP, et al. Exploiting evolutionary steering to induce collateral drug sensitivity in cancer. Nat Commun. 2020;11:1923.
4. Greaves M. Evolutionary determinants of cancer. Cancer Discov. 2015;5:806–20.
5. Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. Carcinogenesis. 2010;31:100–10.
6. Maris JM, Denny CT. Focus on embryonal malignancies. Cancer Cell. 2002;2:447–50.
7. Greaves M. A causal mechanism for childhood acute lymphoblastic leukaemia. Nat Rev Cancer. 2018;18:471–84.
8. Pinkel D. Personal journeys with childhood leukaemia. In: Greaves M, editor. White blood. World Scientific: Singapore; 2008.
9. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943–55.
10. Essig S, Li Q, Chen Y, Hitzler J, Leisenring W, Greenberg M, et al. Risk of late effects of treatment in children newly diagnosed with standard-risk acute lymphoblastic leukaemia: a report from the Childhood Cancer Survivor Study cohort. Lancet Oncol. 2014;15:841–51.
11. Winther JF, Schmiegelow K. How safe is a standard-risk child with ALL? Lancet Oncol. 2014;15:782–3.
12. Ward G. The infective theory of acute leukaemia. Br J Child Dis. 1917;14:10–20.
13. Schulz TF, Neil JC. Leukemia. In: Henderson ES, Lister TA, Greaves MF, editors. Philadelphia: Saunders; 2002.
14. Greaves MF. Speculations on the cause of childhood acute lymphoblastic leukemia. Leukemia. 1988;2:120–5.
15. Mori H, Colman SM, Xiao Z, Ford AM, Healy LE, Donaldson C, et al. Chromosome translocations and covert leukemic clones are generated during normal fetal development. Proc Natl Acad Sci USA. 2002;99:8242–7.
16. Schafer D, Olsen M, Lahnemann D, Stanulla M, Slany R, Schmiegelow K, et al. Five percent of healthy newborns have an ETV6-RUNX1 fusion as revealed by DNA-based GIPFEL screening. Blood. 2018;131:821–6.
17. Maia AT, Koechling J, Corbett R, Metzler M, Wiemels JL, Greaves M. Protracted postnatal natural histories in childhood leukemia. Genes Chromosomes Cancer. 2004;39:335–40.
18. Pannunzio NR, Lieber MR. AID and reactive oxygen species can induce DNA breaks within human chromosomal translocation fragile zones. Mol Cell. 2019;73:639.
19. Papaemmanuil E, Rapado I, Li Y, Potter NE, Wedge DC, Tubio J, et al. RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. Nat Genet. 2014;46:116–25.
20. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007;446:758–64.
21. Ford AM, Palmi C, Bueno C, Hong D, Cardus P, Knight D, et al. The TEL-AML1 leukemia fusion gene dysregulates the TGF-beta pathway in early B lineage progenitor cells. J Clin Investig. 2009;119:826–36.
22. Beneforti L, Dander E, Bresolin S, Bueno C, Acunzo D, Bertagna M, et al. Pro-inflammatory cytokines favor the emergence of ETV6-RUNX1-positive pre-leukemic cells in a model of mesenchymal niche. Br J Haematol. 2020;190:262–73.
23. Cazzaniga G, Bisanti L, Randi G, Deandrea S, Bungaro S, Pregliasco F, et al. Possible role of pandemic AH1N1 swine flu virus in a childhood leukemia cluster. Leukemia. 2017;31:1819–21.
24. Kroll ME, Draper GJ, Stiller CA, Murphy MF. Childhood leukemia incidence in Britain, 1974–2000: time trends and possible relation to influenza epidemics. J Natl Cancer Inst. 2006;98:417–20.
25. Li CK, Zee B, Lee J, Chik KW, Ha SY, Lee V. Impact of SARS on development of childhood acute lymphoblastic leukaemia. Leukemia. 2007;21:1353–6.
26. Greaves M. COVID-19 and childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2020;67:e28481.
27. Urayama KY, Buffler PA, Gallagher ER, Ayoob JM, Ma X. A meta-analysis of the association between day-care attendance and childhood acute lymphoblastic leukaemia. Int J Epidemiol. 2010;39:718–32.
28. Rudant J, Lightfoot T, Urayama KY, Petridou E, Dockerty JD, Magnani C, et al. Childhood acute lymphoblastic leukemia and indicators of early immune stimulation: a Childhood Leukemia International Consortium study. Am J Epidemiol. 2015;181:549–62.
29. Amitay EL, Keinan-Boker L. Breastfeeding and childhood leukemia incidence: A meta-analysis and systematic review. JAMA Pediatr. 2015;169:e151025. https://doi.org/10.1001/jamapediatrics.2015.1025.
30. Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Haematologica. 2010;95:1405–14.
31. Moriyama T, Relling MV, Yang JJ. Inherited genetic variation in childhood acute lymphoblastic leukemia. Blood. 2015;125:3988–95.
32. Lu Z, Xie J, Wu G, Shen J, Collins R, Chen W, et al. Fasting selectively blocks development of acute lymphoblastic leukemia via leptin-receptor upregulation. Nat Med. 2017;23:79–90.
33. Moore RE, Townsend SD. Temporal development of the infant gut microbiome. Open Biol. 2019;9:190128.
34. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, et al. Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. Cell Host Microbe. 2018;24:133–45 e5.
35. Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015;17:690–703.
36. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. Nat Med. 2016;22:713–22.

37. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;336: 1268–73.
38. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013;504:451–5.
39. Burrows MP, Volchkov P, Kobayashi KS, Chervonsky AV. Microbiota regulates type 1 diabetes through Toll-like receptors. Proc Natl Acad Sci USA. 2015;112:9973–7.
40. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nat Rev Immunol. 2017;17:219–32.
41. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. Science. 2016;352:539–44.
42. Schnorr SL, Sankaranarayanan K, Lewis CM Jr., Warinner C. Insights into human evolution from ancient and contemporary microbiome studies. Curr Opin Genet Dev. 2016;41:14–26.
43. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222–7.
44. Ruggles KV, Wang J, Volkova A, Contreras M, Noya-Alarcon O, Lander O, et al. Changes in the gut microbiota of urban subjects during an immersion in the traditional diet and lifestyle of a rainforest village. mSphere. 2018;3:e00193–18. https://doi.org/10.1128/mSphere.00193-18.60.
45. Bach JF. The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. Nat Rev Immunol. 2018;18:105–20.
46. Rook GAW. The hygiene hypothesis and Darwinian medicine. Basel: Birkhauser Basel; 2009.
47. Bridgman SL, Kozyrskyj AL, Scott JA, Becker AB, Azad MB. Gut microbiota and allergic disease in children. Ann Allergy Asthma Immunol. 2016;116:99–105.
48. Vatanen T, Franzosa EA, Schwager R, Tripathi S, Arthur TD, Vehik K, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. Nature. 2018;562:589–94.
49. Siljander H, Honkanen J, Knip M. Microbiome and type 1 diabetes. EBioMedicine. 2019;46:512–21.
50. Rook GA. The hygiene hypothesis and the increasing prevalence of chronic inflammatory disorders. Trans R Soc Trop Med Hyg. 2007;101:1072–4.
51. Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun. 2016;7:12015.
52. Durack J, Lynch SV. The gut microbiome: relationships with disease and opportunities for therapy. J Exp Med. 2019; 216:20–40.
53. Rajagopala SV, Vashee S, Oldfield LM, Suzuki Y, Venter JC, Telenti A, et al. The human microbiome and cancer. Cancer Prev Res. 2017;10:226–34.
54. Tilg H, Adolph TE, Gerner RR, Moschen AR. The intestinal microbiota in colorectal cancer. Cancer Cell. 2018;33:954–64.
55. Weingarden AR, Vaughn BP. Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. Gut Microbes. 2017;8:238–52.
56. Korpela K, Salonen A, Vepsalainen O, Suomalainen M, Kolmeder C, Varjosalo M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. Microbiome. 2018;6:182.
57. Juul FE, Garborg K, Bretthauer M, Skudal H, Oines MN, Wiig H, et al. Fecal microbiota transplantation for primary clostridium difficile infection. N. Engl J Med. 2018;378: 2535–6.
58. DeFilipp Z, Peled JU, Li S, Mahabamunuge J, Dagher Z, Slingerland AE, et al. Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. Blood Adv. 2018;2:745–53.
59. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science. 2018;359:91–7.
60. Kumar H, Collado MC, Wopereis H, Salminen S, Knol J, Roeselers G. The bifidogenic effect revisited-ecology and health perspectives of bifidobacterial colonization in early life. Microorganisms. 2020;8:1855. https://doi.org/10.3390/microorganisms8121855.
61. Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, Chandel DS, et al. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature. 2017;548:407–12.
62. Durack J, Kimes NE, Lin DL, Rauch M, McKean M, McCauley K, et al. Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by Lactobacillus supplementation. Nat Commun. 2018;9:707.
63. Patole SK, Rao SC, Keil AD, Nathan EA, Doherty DA, Simmer KN. Benefits of Bifidobacterium breve M-16V Supplementation in Preterm Neonates - A Retrospective Cohort Study. PLoS One. 2016;11:e0150775.
64. Insel R, Knip M. Prospects for primary prevention of type 1 diabetes by restoring a disappearing microbe. Pediatr Diabetes. 2018;19:1400–6.
65. Wang Y, Xue J, Zhou X, You M, Du Q, Yang X, et al. Oral microbiota distinguishes acute lymphoblastic leukemia pediatric hosts from healthy populations. PLoS One. 2014;9: e102116.
66. Rodriguez-Hernandez G, Hauer J, Martin-Lorenzo A, Schafer D, Bartenhagen C, Garcia-Ramirez I, et al. Infection exposure promotes ETV6-RUNX1 precursor B-cell leukemia via impaired H3K4 demethylases. Cancer Res. 2017;77:4365–77.
67. Marino E, Richards JL, McLeod KH, Stanley D, Yap YA, Knight J, et al. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. Nat Immunol. 2017;18:552–62.
68. Aumeunier A, Grela F, Ramadan A, Pham Van L, Bardel E, Gomez Alcala A, et al. Systemic Toll-like receptor stimulation suppresses experimental allergic asthma and autoimmune diabetes in NOD mice. PLoS One. 2010;5:e11484.
69. Calcinaro F, Dionisi S, Marinaro M, Candeloro P, Bonato V, Marzotti S, et al. Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. Diabetologia. 2005;48:1565–75.
70. Kriegel MA, Sefik E, Hill JA, Wu HJ, Benoist C, Mathis D. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. Proc Natl Acad Sci USA. 2011;108:11548–53.
71. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008;455: 1109–13.
72. Kaila B, Taback SP. The effect of day care exposure on the risk of developing type 1 diabetes: a meta-analysis of case-control studies. Diabetes Care. 2001;24:1353–8.
73. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. N. Engl J Med. 2000; 343:538–43.
74. Malcova H, Sumnik Z, Drevinek P, Venhacova J, Lebl J, Cinek O. Absence of breast-feeding is associated with the risk of type 1 diabetes: a case-control study in a population

with rapidly increasing incidence. Eur J Pediatr. 2006; 165:114–9.
75. Lodge CJ, Tan DJ, Lau MX, Dai X, Tham R, Lowe AJ, et al. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. Acta Paediatr. 2015;104:38–53.
76. Cardwell CR, Stene LC, Joner G, Cinek O, Svensson J, Goldacre MJ, et al. Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. Diabetologia. 2008;51:726–35.
77. Keag OE, Norman JE, Stock SJ. Long-term risks and benefits associated with cesarean delivery for mother, baby, and subsequent pregnancies: Systematic review and meta-analysis. PLoS Med. 2018;15:e1002494.
78. Boiers C, Richardson SE, Laycock E, Zriwil A, Turati VA, Brown J, et al. A human IPS model implicates embryonic B-myeloid fate restriction as developmental susceptibility to B acute lymphoblastic leukemia-associated ETV6-RUNX1. Dev Cell. 2018;44:362–77 e7.