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Review article

Could peripartum antibiotics have delayed health consequences for the infant?

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Antibiotics are increasingly prescribed in the peripartum period, for both maternal and fetal indications. Their effective use undoubtedly reduces the incidence of specific invasive infections in the newborn, such as group B streptococcal septicaemia. However, the total burden of infectious neonatal disease may not be reduced, particularly if broad-spectrum agents are used, as the pattern of infections has been shown to alter to allow dominance of previously uncommon organisms. This area has been relatively understudied, and there are almost no studies of long-term outcome. Recent findings suggest that such long-term data should be sought. First, there is evidence that organisms initially colonising the gut at birth may establish chronic persistence in many children, in contrast to prompt clearance if first encountered in later infancy, childhood or adulthood. Second, there is a rapidly advancing basic scientific data showing that individual members of the gut flora specifically induce gene activation within the host, modulating mucosal and systemic immune function and having an additional impact on metabolic programming. We thus review the published data on the impact of perinatal antibiotic regimens upon composition of the flora and later health outcomes in young children and summarise the recent scientific findings on the potential importance of gut flora composition on immune tolerance and metabolism.

Keywords Allergy, antibiotics, immunology, intestinal flora.

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Background

The developed world has witnessed a substantial increase in the incidence of allergic and autoimmune disease in young children over the past three decades.[1–3] Studies of the demographics of allergic sensitisation led to postulation by Strachan of the 'hygiene' or 'clean child' hypothesis, which suggests that immune dysregulation in childhood occurs as a result of decreased microbial exposure in early childhood.[4] This hypothesis has subsequently been extended to encompass other immune-mediated pathologies, notably autoimmunity.[5–7] While studies of the clean child hypothesis initially focused on reduced overall infectious exposures in late infancy and early childhood, there has been more recent recognition of a specific role for the commensal bacteria of the gut flora in priming mucosal and systemic immune tolerance in early infancy.[8–11] Administration of probiotics to newborn infants has been reported to reduce the incidence of later allergic disease.[12–15]

Quite dramatic changes have occurred in the pattern of initial colonisation of the gut in the first days of life in infants from developed world countries, and there is an evidence that these changes may be linked to the later development of allergic disease, at both the individual and the national level.[10,13] Murine studies have also demonstrated that changed early-life gut flora may promote the development of autoimmunity in genetically predisposed animals.[1] Among the factors potentially modulating initial colonisation patterns, the use of peripartum antibiotics has become particularly prevalent in modern obstetric and neonatal practice and has led to change in the pattern of neonatal infections.[16–20] We thus aim to explore the mechanisms by which acquired abnormalities in early-life bacterial colonisation may affect the development of tolerance and to review currently available data on the links between perinatal antibiotic administration and later immunopathology.

Normal and abnormal neonatal gut flora

At birth, the neonatal intestine is usually sterile. Maternal vaginal and faecal flora provides the natural first sources of colonising organisms for the neonatal gut. Nature has facilitated this in several mammalian species by ensuring that

infants are normally born vaginally and in the mouth to anus position.[21] In vaginally delivered full-term infants, *Escherichia coli*, *Enterobacteria, Enterococci, Streptococci* and *Staphylococci* are usually present on the first day, with *Bacteroides* in a few.[22] By the end of the first week, *Bifidobacterium* species predominate in breastfed infants, although differences are seen between breastfed and bottle-fed infants. The type of colonising bacteria may be important in immunological maturation: thus, the presence of *Bacteroides fragilis* results in a greater immunoglobulin (Ig) A and IgM production than if absent.[23] Differences in the colonising flora were identified between infants destined to become allergic and those who are non-allergic, suggesting that very early changes in colonising flora may have later effects.[24] Nursing away from the mother on the first night of life, clearly an indirect proxy for likely colonisation by nonmaternal organisms, was, however, associated with the later development of atopy in the 1970 UK Birth Cohort.[25] Thus, there is some, admittedly quite limited, evidence that abnormalities in colonisation may have later effects.[12,14,15,23–25]

There is much stronger evidence that choice of antibiotics for perinatal use may have significant effects on the developing flora. Use of peripartum ampicillin as prophylaxis against group B *Streptococcus* (GBS) infection resulted in a significant reduction in early-onset neonatal GBS infection in the USA, but at the price of a significant increase in resistant coliform infection.[17] While this phenomenon has so far been restricted to preterm and low birthweight infants, it has prompted continued surveillance and reassessment of current GBS prevention strategies.[18,26] In a UK study, use of antepartum coamoxiclav for attempted prolongation of pregnancy, in mothers who ruptured their membranes or laboured before 36 weeks of gestation, was associated with an increase in proven or suspected necrotising enterocolitis.[19] These studies suggest that antibiotics given to mothers do alter the developing indigenous flora in the acute term, and the 'replacement flora' may not be less harmful than those intended for eradication. Both ampicillin and cephalosporins, given to mothers for caesarean section prophylaxis, altered endocervical flora, causing overgrowth of potential pathogens.[20] Widespread use of broad-spectrum antibiotics within a maternity unit will also increase local persistence of antibiotic-resistant organisms and favour nosocomial transmission.[27] This may increase the risk of significant infections for infants born subsequently and make their management more difficult. However, there have been very few studies that have considered the broader long-term implications of such antibiotic-induced changes in patterns of early colonisation. We consider that there is now sufficient evidence from experimental work in animals,[28–31] and the few long-term outcome studies of colonisation in human infants,[12,14,15,23,24,32] to suggest that this is an area that requires proper scientific evaluation.

The importance of bacteria in programming immune development

Symbiotic interactions between the colonising bacteria and the host

The first colonisation of the intestine is one of the most profound immunological exposures faced by the newborn infant. The initial colonising organisms establish a two-way crosstalk with the infant's intestine, inducing gene expression in both the epithelium and the immune system, which establishes a niche, allowing long-term colonisation, probably as part of a biofilm located within the luminal glycocalyx.[28–31] Thus, while probiotics administered after 6 months to human infants have very limited persistence of weeks at most,[33,34] those given perinatally have been shown to persist in some infants for months or years.[12,14,15] Such advantage to early-colonising organisms is thought to occur because these bacteria induce the expression of multiple genes within the intestinal epithelium,[28] remarkably even angiogenetic factors that alter the structure of the villi.[35] Animals maintained germ free require one-third more calories than colonised animals for normal growth and maintain abnormal crypt: villous architecture with reduced epithelial proliferation.[28–31] In human infants, there are similar differences in villous architecture and epithelial proliferation between the intestine of the newborn and of the 3-month-old infant.[36] There is thus a true symbiosis between gut bacteria and their host. However, different bacteria induce expression of different genes within the host's intestinal epithelium: for example, initial monocolonisation of mice by *Bacteroides thetaiotamicron* induced a 200-fold increase in epithelial expression of the gene encoding small proline-rich protein SPRR-2a, a molecule recently implicated in mucosal allergic responses,[37] while *Escherichia coli* had no effects on its expression.[28] Amongst the most important induced epithelial genes for establishing long-term colonisation are the fucosyl transferases, which determine the pattern of glycosylation of the luminal glycocalyx, thus providing selective advantage for adherence of the initial colonising bacteria.[28–31] This advantage can manifest very early in life, and classic reports on human infants showed that *Staphylococci* colonising on the first day of an infant's life were able to prevent others from establishing a niche on the second and third days of life.[38] Changes in initial colonisation patterns may thus have long-term consequences in terms of bacterial persistence.

Host immune responses to bacterial colonisation

The changes induced by initial colonisation are not limited to bacterial persistence and epithelial gene expression. The gut-associated lymphoid tissue represents the largest lymphoid organ in the body. Profound changes occur in the nascent mucosal immune system upon colonisation, including rapid expansion of intraepithelial lymphocyte and Peyer's patch

populations.[39] The development of oral tolerance, a systemic hyporeactivity to ingested antigen central to health, is impaired in the absence of gut bacteria.[40] A specific polysaccharide expressed by *Bacteroides fragilis* directs not only mucosal immune development but also the architecture of peripheral lymphoid organs and systemic immune responses in mice.[41] In its absence, splenic architecture is abnormal (identical to the abnormalities seen in germ-free animals), peripheral lymphocyte responses are skewed towards T_H2 (as seen in allergy) and B-cell follicular thymic hyperplasia develops in the second year.[41] Thus, the symbiosis between host and colonising flora extends to the development of mucosal tolerance and systemic immune priming.

Immune sensing of luminal and mucosally adherent bacteria is mediated by dendritic cells. This occurs either in the organised lymphoid tissue of the Peyer's patches, from where bacteria may also be carried within dendritic cells to mesenteric lymph nodes, or away from organised lymphoid tissue, as dendritic cells can interdigitate between enterocytes and extend processes into the lumen.[42,43] Dendritic cell function can also be modulated at the precursor stage by bacterial or viral exposure, which can lead to a fixed phenotype skewed towards T_H1 or T_H2 responses for the lifetime of the cell, and even in daughter cells after replication.[44] Thus, the first infectious exposures of the nascent immune system shortly after birth may be of much greater importance to immune development than later exposures.

The role of such innate immune responses in directing adaptive immune responses within the gut is much greater than previously recognised, and pattern-recognition molecules on (and within) innate immune cells and the enterocytes themselves are critical in determining eventual immunological outcome. These pattern-recognition molecules—notably toll-like receptors (TLRs) and Nod proteins—play a key role, not only in host defence but also in immunological tolerance.[13,45] The signals transduced by these pattern-recognition molecules, each recognising different bacterial, fungal or viral components, give a relatively stereotyped proinflammatory response, centred on the regulatory molecule nuclear factor-κB (NF-κB).[45] As a consequence of gut colonisation, and the innate immune responses evoked by these bacteria, the mucosal immune system is maintained in a state of 'physiological inflammation', which is required for normal immune tolerance.[8] Blockade of this physiological inflammatory response to the gut flora indeed has been shown to induce sensitisation to dietary antigen.[46] In addition, generation and function of regulatory lymphocytes in mice are directly modulated by signalling through TLR-2.[47] Thus, the gut flora plays a critical role in the priming of immune tolerance. Broadly speaking, there are two ways in which this flora-driven immune programming may be disrupted—by either alterations in the colonising flora in early life or reduced host immune reactivity.

Polymorphisms in many of these pattern-recognition receptors have been reported, giving either an enhanced or a blunted response to microbiological components, likely to have been selected during evolution because these altered responses gave survival advantage against specific pathogens (similarly, high tumour necrosis factor-α producers are favoured where tuberculosis and schistosomiasis are endemic but have higher mortality should they encounter falciparum malaria).[48] However, the disease associations of these polymorphisms in children and young adults of the developed world are now largely with allergic, autoimmune and inflammatory diseases. Thus, loss of function mutations in Nod2 or TLR4 predispose to inflammatory bowel diseases (IBD), while polymorphisms in individual TLRs are associated with atopic and autoimmune diseases.[48–54] An inadequate innate immune response to the flora may thus represent a sensitising event.

Responses to the flora and the development of regulatory lymphocytes

The basis of the 'hygiene' hypothesis is now thought to relate to failed induction of regulatory immune responses in circumstances of abnormal early-life infectious exposure, rather than the straightforward deviation of T_H1/T_H2 balance previously postulated.[8,55] Regulatory T-cell (T_{REG}) populations include T_H3 cells, which produce transforming growth factor-β (TGF-β), a cytokine also key for mucosal IgA responses, and T_R1 cells, which produce interleukin (IL)-10 and $CD4^+CD25^+$ T cells.[56] The transcription factor Foxp3 is the master regulator of T_{REG} production,[55] and the importance of regulatory cells for normal immune development in humans is shown by the severe multifocal inflammatory disease in children with mutations in this gene (IPEX syndrome).[57] The few studies on regulatory lymphocytes in children show a clear association between a reduced T_{REG} response and allergic sensitisation. Reduced IL-10 responses were associated with allergic sensitisation in a developing world population (Gabon),[58] while impaired generation of mucosal T_H3 cells was detected in a population of UK children with multiple food allergies.[59] Importantly, recovery from cow's milk allergy was associated with restoration of $CD4^+CD25^+$ T_{REG} populations.[60]

The relevance of these findings to perinatal antibiotic therapy is that generation of regulatory lymphocytes within the intestine requires an obligatory input from the gut flora into the innate immune system. This was confirmed in transgenic mice whose only T cell recognised hen egg lysozyme.[46] Remarkably, they were able to eat this protein without problems, unless response to the gut flora by the innate immune system was disrupted—tolerance was then abrogated and intestinal inflammation supervened. It is notable that abnormal immune responses to normal colonisation leads to mucosal inflammation or systemic autoimmunity in numerous animal models of IBD[8] and that many inherited human immunodeficiencies are characterised by intestinal immunopathology,

allergy and autoimmunity.[61] Thus, the generation of regulatory lymphocytes may be impaired because of either inborn abnormalities in the host's immune system or acquired problems with the process of colonisation. In human infants, the former situation is thankfully rare. However, the latter situation is not, and many infants are now becoming colonised by bacterial patterns probably unique in evolutionary history. Functional polymorphisms in pattern-recognition molecules of the innate immune system, of previous selective advantage, may ensure that an inadequate tolerising response may be made in such circumstances.

Modulatory effects of breastfeeding on colonisation and immune responses

It is important to note that breastfeeding has important effects on colonisation patterns and may also have specific effects on immune responses in the infant.[62] Breastfed infants generally become colonised with higher numbers of bifidobacteria and lactobacilli, at least partly due to the prebiotic activity of complex carbohydrates within breast milk.[63] Both colostrum and breast milk contain lactoferrin, which may restrict growth of pathogens. In addition, breast milk contains immunoregulatory cytokines such as TGF-β and lymphocytes expressing gut-homing markers.[64] There is admittedly yet no firm evidence that breastfeeding reduces the overall incidence of allergy or autoimmune disease,[63] but the effects of breastfeeding on limiting antibiotic-induced perturbation of colonisation provide one further argument in strong support of 'baby-friendly' breastfeeding policies.

Abnormal host–flora interaction in early infancy may induce immunopathology

Proof of principle that abnormal responses to the flora in very early life may predispose to later immunopathology was provided in a transgenic mouse model of IBD, in which the T-cell receptor was mutated.[65] Animals maintained germ free did not develop disease in this model, confirming the obligatory role of the normal flora. However, excision of mucosal lymphoid tissue by appendicectomy also completely prevented disease in this model, provided it was performed before in infancy—later excision was completely ineffective.[65] Thus, sensitisation to the flora occurred within the first month of life in this model, leading to later disease. An important feature of this model is the period of apparent wellbeing in these animals in early life, equivalent in human terms to several years.[65] Aberrant responses to the flora causing neonatal sensitisation may therefore be clinically silent initially and the true causative link thus likely to remain unsuspected outside the experimental situation.

Changed patterns of early-life gut colonisation may also determine later systemic autoimmunity. In the nonobese diabetic mouse, a move from conventional housing to a strict pathogen-free environment induces a 50% increase in the incidence of diabetes, within one generation.[1] These remarkable findings further underline the importance of establishing appropriate gut flora in early life and of making an appropriate immune response. They also demonstrate that adverse consequences may not be predictable or indeed even recognisable in the early stages.

Abnormal host–flora interactions and metabolic programming

There are additional, potentially lifelong, effects on the developing infant, mediated by the bacteria initially colonising the intestine. Again, most of the proof-of-principle studies have been murine studies; hence, some caution is required in interpretation.[66]

Programming of the hypothalamopituitary axis (HPA) in early murine infancy was shown to be dependent upon the normal gut flora and significantly exaggerated HPA responses seen in germ-free animals or those colonised with abnormal flora in infancy, in contrast to those in a strict pathogen-free environment.[67] These changes, which included reduced cortical and hippocampal brain-derived neurotrophic factor expression in addition to plasma adrenocorticotrophic hormone and corticosterone responses, could be reversed by colonisation with *Bifidobacteria infantis*, an important member of the normal infant flora. However, monoassociation with *Escherichia coli* had the opposite effect of increasing HPA priming abnormality, in a manner dependent on binding to the epithelium (this effect was abrogated by targeted deletion of intimin genes required for epithelial tropism and adherence).[67] Not only did aberrant colonisation have long-term effects on HPA response but this could only be reversed by administration of normal bacteria within a very narrow time window in early infancy. This finding of a critical period in very early life, for long-term metabolic programming by the flora, is similar to that seen for immune sensitisation, giving further support to the concept that responses to the gut flora in early infancy are critical for long-term health and that disturbance of the intestinal flora in early life may silently promote later disease.

The first bacteria to colonise the intestine also induce a long-term change in nutrition, and different bacteria have potentially different effects.[28–31] Thus, *Bacteroides thetaiotamicron* has a remarkable ability to breakdown plant polysaccharides due to production of more than 170 glycosylhydrolases. Colonisation of the intestine with this organism induces symbiotic expression of epithelial monosaccharide transporters.[28] Of potential long-term public health concern, colonisation of the germ-free intestine in mice also had profound effects on adipogenesis, potentially contributing to obesity and insulin resistance.[68] The enhanced fat deposition in these mice was mediated through inhibition of the constitutive epithelial production of fasting-induced adipocyte factor, which functions

as a circulating lipoprotein lipase inhibitor. Colonisation increased circulating leptin levels, as well as glucose and insulin, and also promoted insulin resistance. Although the individual bacteria determining these metabolic effects were not determined, monocolonisation with *Bacteroides thetaiotamicron* had a much reduced effect.[68] More work is clearly needed to determine individual bacterial contributions, but the implication for paediatric practice is that perturbation of evolutionarily determined colonisation patterns may have very significant long-term metabolic implications beyond those previously ascribed to the gut flora.

An alternative viewpoint—could potentially allergic infants be more susceptible to perinatal infection?

While we make the case that the use of broad-spectrum antibiotics may have longer term implications than previously considered, it is also important to consider whether infants genetically destined to be allergic may be at increased risk of acquiring perinatal infection and thus being given antibiotics in the first place. This argument would presumably not apply to the many infants exposed to antibiotics for maternal indications alone, unless chorioamnionitis or rupture of membranes was promoted by maternal allergic predisposition. There is no evidence of this and indeed none that that allergy is associated with other invasive bacterial disease in adults. In addition, the pathogenesis of membrane rupture is dependent on an enhanced proinflammatory response of broadly T_H1 type,[69] and would appear counter to the classic T_H2 response in allergy.[1,3,4,10] Among term infants receiving postnatal screening and antibiotics for potential infection (approximately 12% of all deliveries[70]), only around 3% of these are finally diagnosed with culture-proven or culture-negative sepsis.[70] Thus, although screening and treatment are usually clinically necessary, more than 95% of screened infants (>10% of total births) receive antibiotics for what turn out to be noninfectious causes. Genetic predisposition to allergy is unlikely to be of relevance to this group.

For the fewer infants treated for genuine sepsis, it is possible that inherited innate immune responses predisposing to allergy might increase susceptibility to invasive bacterial disease. Loss of function polymorphisms in several TLRs is associated with allergy,[48–53] and a blunted NF-κB-mediated proinflammatory response to bacterial exposure might then result, favouring invasive disease. Expression of TLR-2, known to be associated with asthma and severe eczema,[51,52] is reduced on neonatal monocytes in comparison with adult cells.[71] TLR-2 expression is particularly reduced in cord blood monocytes of children born to allergic mothers in comparison with that in monocytes from their own mothers and from infants born to nonatopic mothers.[72,73] This is functionally important as monocytes from such allergic infants show lower IL-6 responses to peptidoglycan than do those from infants with nonatopic mothers.[73] It is notable that deficiency of TLR-2 in mice predisposes to invasive infection following exposure to gram-positive organisms including *Staphylococcus aureus*[74] and GBS,[75] although protecting from lethality in invasive GBS infection through reduced cytokine responses.[75] Links between atopic predisposition and invasive perinatal infectious disease clearly warrant further study but are not relevant to the great majority of infants exposed to antibiotics in the perinatal period.

Broad-spectrum antibiotics in infancy may induce immunopathology

A particularly potent means of disturbing early-colonisation patterns is by using antibiotics with a broad and unselective spectrum. A specific role for broad-spectrum antibiotics in early life, as a determinant of allergic sensitisation, has been provided by animal studies. Administration of kanamycin to 3-week-old mice reduced Peyer's patch cellularity and induced skewing of immune responses towards T_H2 (increased IgE and IgG1 and stimulated IL-4 production) while reducing T_H1 responses (interferon-γ production).[76] These changes could be reversed by colonisation with *Enterococcus faecalis* and attenuated by *Lactobacillus acidophilus* but were exacerbated by *Bacteroides vulgatus*.

Additional studies of broad-spectrum antibiotic administration in older mice have identified a potential role for induced candidal overgrowth in promoting airway allergy. The first evidence confirming the role of antibiotics in airway allergic disease came in a study in which mice were treated with a short course of cefoperazone, followed by a single gavage of Candida and developed a CD4-mediated pulmonary allergic response upon subsequent challenge with mould spores.[77] The pulmonary response was characterised by recruitment of eosinophils and mast cells and manifested enhanced IgE and both T_H1 and T_H2 cytokine responses. These studies were replicated in a different mouse strain, and using the dietary antigen ovalbumin rather than mould spores, with identical effects.[78] Thus, the effects of antibiotics appeared independent of host genetics and antigen specificity and were characterised by mucosal allergy on later challenge.

Evidence that antibiotics may affect immunological health in the long term in humans

Most of the data concerning antibiotic effects on allergic sensitisation in human infants have looked retrospectively at antibiotic doses during the first year of life and have relied upon parental recall. Cross-sectional studies have suggested a positive association between antibiotic dosage in infancy and later allergic disease.[79–82] However, results of longitudinal studies have been conflicting, and many have failed to

confirm a causative link.[83–86] Two factors suggest that these findings may not be applicable to the perinatal period. First, it is clear that changes in composition of the flora are much more short-lived outside the perinatal period—as demonstrated by the very brief persistence of probiotics given to 10-month-old infants[33,34] in contrast to their long-term carriage when given to neonates.[12,14,15] Second, it is important to recognise the potential confounding fact that children destined to be allergic may have increased propensity for recurrent viral infections, leading to increasing antibiotic prescription. A pattern of low IgA, IgG subclasses, CD8 and Natural Killer cells is associated with prolonged viral infections in infancy in food allergic infants.[87,88] Thus, there is a significant potential confounding of genetic susceptibility with antibiotic effects in study of older infants. Two longitudinal studies concluded that frequent antibiotic use in infancy was more common in those children destined to be asthmatic, rather than causative in itself.[89,90]

Some preliminary evidence for an effect of antibiotics upon later allergic disease when given peripartum comes from the West Midlands General Practice Research Database, a cohort of 24 690 infants who were evaluated for incidence of hay fever, asthma, wheeze and eczema.[91] Approximately one-third of mothers were prescribed one or more courses of antibiotics during pregnancy. Asthma prevalence increased in a dose-dependent manner, with number of antibiotic courses given antenatally, and the same was found for hay fever and eczema. By contrast, analysis of 5519 members of the 1970 British Cohort study did not identify increased hay fever at 26 years of age in those infants reported to receive prophylactic antibiotics in the first few days of life.[25] These are clearly very preliminary data, and the paucity of studies of long-term outcome of perinatal antibiotic use is striking. While there is an emerging evidence that probiotic use in infancy and early life may have long-term consequences on health,[12–15,92] the much more common scenario of antibiotic administration to mother or newborn infant remains almost completely unstudied in anything but the short term.

Summary

There are separate data from human and animal studies demonstrating that perinatal antibiotics may affect the gut flora and that the early-life flora is associated with allergic sensitisation, metabolic priming and development of regulatory lymphocyte populations. However, there is an almost complete lack of studies that have attempted to link these common phenomena in an assessment of potential causality or basic mechanism. There is rapidly increasing evidence from experimental studies that the initial colonisation of the intestine is a moment of pivotal importance in long-term health, playing a profound role in imprinting of immune and systemic homeostasis. It has been suggested that Barker's hypothesis of fetal predestination of disease should be expanded to encompass initial colonisation,[13] and the more recent murine studies support this contention. The potential for long-term persistence of early-colonising bacteria suggests that much more thought should be given to the late consequences of perinatal broad-spectrum antibiotics. As a minimum, more studies are needed on the bacteriological and immunological consequences of antibiotic administration to neonates. Focusing on short-term antibacterial efficacy alone appears desperately short-sighted, and we believe unacceptably so in the face of a comprehensive body of data from the direction of basic science. The finding that specific molecules of individual bacteria have defined critical roles in immune development[37] suggests that the flora should no longer be considered an amorphous entity and that more thought needs to be given to its composition during the critical times of immune priming in early life. While that mandates much more basic work, it also suggests more practically that the classic microbiological dictum, of never using a broad-spectrum antibiotic when a narrow-spectrum one will do, has probably a unique resonance for the perinatologist. ■

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