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The influence of the microbiota on type-1 diabetes: on the threshold of a leap forward in our understanding

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Summary: The last several years have seen breakthroughs in techniques to track the symbiont communities that normally colonize mammals (the microbiota) and in cataloguing the universe of the genes they carry (the microbiome). Applying these methods to human patients and corresponding murine models should allow us to decipher just how the microbiota impacts type-1 diabetes, i.e. which particular microbes are responsible and the cellular and molecular processes that are involved. Here, at its threshold, we set the stage for what promises to be an exciting rejuvenated area of investigation.

Keywords: *autoimmune disease, type-1 diabetes, microbiota, intestine, bacteria*

Introduction

It has long been recognized that initiation and progression of the common autoimmune diseases reflect the influences of a complex, interacting network of genetic and non-genetic factors (1). The last 10 years might be considered the field's 'genetics decade', as modern genetic approaches were applied to the identification of heritable elements impacting a variety of autoimmune disorders (2). As discussed below, the next 10 years promise to be the 'decade of the microbiome', driven by impressive advances in next-generation sequencing methods and bioinformatics deconvolution techniques, as well as a more sophisticated appreciation of the molecular and cellular players driving normal and pathogenic immune responses.

The focus of this review is autoimmune diabetes [type-1 diabetes (T1D)]: today, at the threshold of burgeoning interest, what do we know about the impact of microbial symbionts on the onset and course of T1D? Of necessity at this point, we concentrate on information derived from murine diabetes models. We provide an overview of T1D pathogenesis, briefly summarize the vision of non-genetic influences on T1D obtained via classical approaches, discuss our new appre-

ciation of the microbiota and microbiome, emphasize recent data on the interplay between the microbiota and immune system, and finish with a presentation of recent data on the influence of the microbiota on T1D.

Autoimmune diabetes in patient and models

T1D, characterized by specific destruction of the insulin-producing β cells of the islets of Langerhans of the pancreas, is a classic example of an organ-specific autoimmune disease (reviewed in 3). It unfolds in two stages: an occult phase, termed insulitis, when a mixed population of leukocytes invades the islets, eventually provoking specific destruction of the β cells; and an overt phase, diabetes, when the bulk of β cells have been destroyed or disabled, and insulin production is no longer sufficient to regulate blood-glucose levels, resulting in hyperglycemia. Although T1D is an ancient and increasingly frequent disease, we remain surprisingly ignorant concerning its etiology and pathogenesis. We do not know what triggers it, have only a rudimentary understanding of the factors that regulate its progression, and have a confused view of the final effector mechanism(s). Consequently, we still do not know how to prevent or reverse autoimmune diabetes in a sufficiently innocuous manner to be therapeutically useful. Faced with the difficulties of addressing these issues in humans, many investigators have turned to small-animal models, in particular the non-obese diabetic (NOD) mouse strain and simplified derivatives of it, notably T-cell receptor (TCR) transgenic (tg) lines skewed for diabetogenic TCR specificities (4–6).

Developed in the late 1970s, the NOD mouse strain is currently the most commonly used animal model of T1D (reviewed in 7). Disease develops spontaneously in NOD mice, sharing several critical features with the human disorder. As is typical (though not universal) in humans, the course of pathology is protracted: insulitis begins at 4–6 weeks of age, but diabetes is not evident until 15–30 weeks in most NOD colonies. Also like the human disease, diabetes in these animals is primarily T-lymphocyte-mediated, although other cell types do play a role. Furthermore, the human and mouse disorders are both under complex polygenic control, by far the most important contributor being the human leukocyte antigen (HLA)/major histocompatibility complex (MHC) genomic segment. The diabetes-associated HLA/MHC class II molecules in the two species show common structural features, which differ from those of class II molecules not associated with disease (8). Genetic dissections also revealed commonalities in several other immunological pathways, e.g., costimulation, interleukin-2 (IL-2) signaling (9). Lastly, and most importantly, an analysis of the literature indicated that the effects of immunomodulatory treatments in NOD mice are nicely predictive of responses by T1D patients when analogous interventions – with comparable injection doses, timing, and routes – are performed (10).

T1D is primarily a T-cell-mediated disorder

Roles for several cell types in NOD diabetes pathogenesis have been documented, including T cells, B cells, natural killer cells, macrophages (MFs), and dendritic cells (DCs). However, a battery of studies has clearly established that T lymphocytes are central to disease initiation and progression in both mice and humans (reviewed in 7). In the NOD and other mouse models, most of the leukocytes in the islet infiltrate are T cells, and T-cell autoreactivity to β cells is readily demonstrable. Disease does not develop in NOD mice genetically athymic or T lymphopenic or in animals thymectomized at birth; likewise, it is dampened or even abrogated by reagents that interfere with T-cell function. Finally, diabetes can be transferred by injecting T cells from diseased donors into healthy NOD recipients, the simplest demonstration being inoculation of a single T-cell clone into a lymphocyte-deficient NOD mouse. There is ample indication that the human disease is also mediated by T lymphocytes: patients exhibit similar islet histology, T-cell autoreactivity to β-cell antigens, and positive response to treatment with T-cell inhibitors (reviewed in 3).

It has long been recognized that both $CD4^+$ and $CD8^+$ T lymphocytes are important effector populations in the unfolding of NOD diabetes (reviewed in 7). $CD4^+$ T cells are the dominant islet invaders through much of disease progression, but $CD8^+$ T cells are required both to effectively initiate insulitis and to optimally destroy β cells. Interferon-γ (IFN-γ)-producing T-helper 1 (Th1) cells are thought to be crucial effectors, although genetic support for this notion has been equivocal, with both infirming (11, 12) and confirming (13) data. A potential role for Th17 cells has been more controversial, with reports that either IL-17 or Th17 cells can promote (14–16) or protect from (17, 18) NOD diabetes. Potential complications in interpreting these contradictory results are that Th17 cells, especially after transfer into lymphopenic recipients, can convert to IFN-γ-producing Th1-like cells (19, 20), that Th17 cells produce cytokines other than IL-17, that IL-17 is made by cells other than Th17 cells (14), and that IL-17's role may vary with disease state, e.g. stage or severity (15, 16). It has also been known for a long time that T-regulatory

(Treg) cell populations can exert substantial influences on the progression of NOD diabetes (reviewed in 21–23). Primary among these is the Foxp3$^+$CD4$^+$ Treg subset. The extensive evidence of these cells' ability to rein in pathogenesis in NOD (and other) mice, and suggestively similar descriptions of an analogous regulatory population in humans, has fueled energetic efforts to harness Tregs for therapy of autoimmune diabetes (22).

T1D has a regulated progression

BCD2.5, which carries rearranged *Tcra* and *Tcrb* transgenes derived from a diabetogenic CD4$^+$ T-cell clone isolated from a NOD mouse, is a commonly used TCR tg model of T1D (4). One of the most important observations that came from studies on BDC2.5 is that autoimmune diabetes is a regulated disease, with distinct junctures (24) (Fig. 1). The first control-point (checkpoint 0) is the appearance of β-cell-reactive T cells in the peripheral lymphoid organs; this feature distinguishes NOD mice from strains that are not diabetes-prone (25). Another control-point (checkpoint 1) is evident at the initiation of insulitis: even though BDC2.5 mice have abundant β-cell-reactive T cells from shortly after birth, there are no overt signs of autoreactivity until around 3 weeks of age, when insulitis begins, abruptly and intensely. A last point of control (checkpoint 2) manifests at the transition from insulitis to diabetes: BDC2.5 animals show insulitis at 3 weeks, and it is rampant by 8 weeks, but diabetes does not show up until months later.

These various checkpoints are also apparent in the regular NOD strain but are less synchronized and therefore less distinct. It was thought that the pauses before the initiation of insulitis and between the development of insulitis and diabetes in NOD mice merely represented the time it takes for adequate numbers of β-cell-reactive T cells to be recruited and to expand sufficiently to exert a discernible effect; however, this is clearly not the case in the TCR tg mice, which are born with high numbers of autoreactive T cells. The same checkpoints may also exist in human diabetes, at least in its most frequent form: anti-β-cell autoantibodies and metabolic abnormalities (as manifestations of insulitis) are not typically detected until a few years of age, and many more years may pass before the overt manifestation of diabetes (26).

The concept of regulated checkpoints in T1D progression has proven to be a useful framework for constructing a comprehensive disease scenario, aiding in sorting the influences of a sometimes bewildering set of effector and regulatory elements.

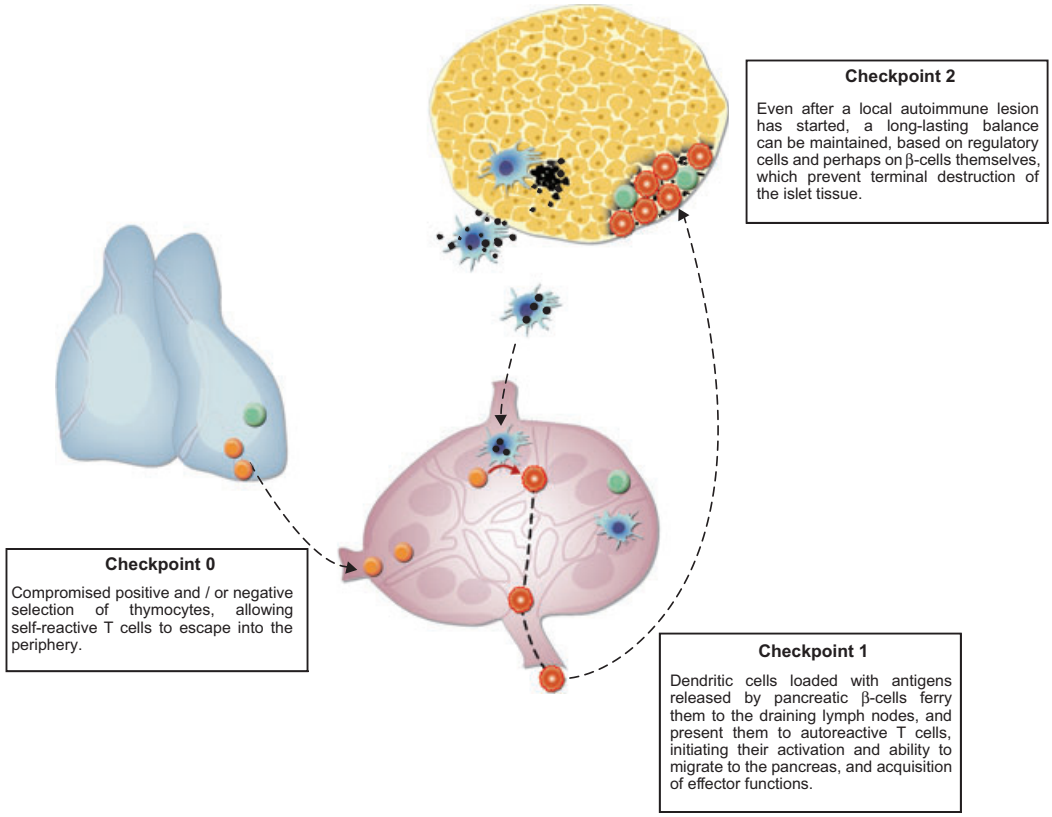

***Fig. 1.* Checkpoints in the development of type 1 diabetes.**

Environmental influences on autoimmune diabetes

As is the case for most autoimmune diseases, the low concordance rate of T1D in monozygotic twins (<40%) argues for the importance of non-genetic influences on the initiation and/or progression of this disorder (reviewed in 27). The immediate assumption has usually been that the deviation from complete concordance reflects environmental effects. Several epidemiological observations support this notion: (i) a north–south cline in development of T1D in European Caucasians; (ii) an augmented frequency in individuals who migrated from a low-incidence to a high-incidence environment (at a sufficiently young age); (iii) a several-fold increase in the frequency of T1D in multiple geographic regions since the 1950s; and (iv) a striking recent elevation in the number of children <5 years of age diagnosed with this malady. It is also well established that non-genetic influences modulate disease kinetics and penetrance in rodent models of autoimmune diabetes (reviewed in 7). The most influential environmental factors impacting T1D are considered to be microbes (e.g. symbiont bacteria, enteroviruses, infectious agents), diet (e.g. cow's milk, omega-3 fatty acids), and toxins. A favored explanation, drawing from similar data on other immunological diseases (both allergic and autoimmune), evokes the 'hygiene hypothesis' of Strachan (28), as modified by Wills-Karp *et al.* (29): the increase in diabetes incidence may reflect a reduction in childhood infections, thought to be essential for conditioning the immune system in preparation for a range of later challenges. It is hoped that the ongoing The Environmental Determinants of Diabetes in the Young study, designed to evaluate how a range of environmental factors impinge on the unfolding of T1D, will elucidate such disease-modulating influences.

Environmental influences are not the only explanation for the non-concordance of disease in identical twins, especially in the case of immunological disorders. The fact that a varying fraction of mice develop clinical diabetes is a good illustration of this point. Stochastic elements are one alternative explanation. As alluded to above, T1D in patients and models depends on both T and B lymphocytes. These cells' antigen-specific receptors are generated via random rearrangement of *Tcr* and *Ig* genes, so even identical twins have different lymphocyte repertoires. It is possible that a non-diabetic, non-concordant twin simply does not produce a potent enough repertoire of diabetogenic specificities to trigger/sustain islet β-cell attack or a competent enough repertoire of regulatory specificities to keep the attack in check. Another relevant stochastic process is generation of the Aire-dependent repertoire of peptides presented by medullary epithelial cells (MECs) in the thymus, which is critical for clonal deletion of autoreactive T-cell clones (30). That this process might have importance in the diabetes context is supported by the genetic association between a reduced incidence of T1D and alleles of the *Ins* gene that promote insulin expression in MECs (31). A second alternative to environmental explanations for twin-non-concordance is epigenetic factors, and this area is garnering growing interest (32). It has already been demonstrated that maternal undernutrition during pregnancy can influence metabolic parameters (such as insulin sensitivity) in first- and second-generation offspring of mice (33). We are just beginning to learn about like effects on T1D and about underlying molecular changes in DNA and/or chromatin, but it is noteworthy that resveratrol, an activator of the type III histone deacetylase, sirtuin1, could prevent and treat diabetes in NOD mice (34), and the oral histone deacetylase inhibitor, ITF2356, normalized streptozotocin-induced hyperglycemia in mice (35).

Obscuring the borders of several of these influences on diabetes susceptibility is the effect of the microbiota. The impact of the constellation of symbiont microbes hosted by a mammal, in particular those residing in the gut, can be thought of as environmental because their representation reflects the surroundings, stochastic because random processes seem to shape host colonization (36) or epigenetic because the microbiota is primarily transmitted from the mother to her offspring during birth. Our understanding of the effects of the microbiota on T1D is in its infancy, but it seems worthwhile to summarize current knowledge to set the state for future exploration.

The universe of symbiont microbes

Mammals host trillions of microbes at diverse sites in the body, in particular in the intestinal tract (reviewed in 37–40). It has been estimated that an adult human carries over 10 times more 'foreign' microbial cells in the gut than 'self' somatic and germ cells in the entire body. In the past, it was difficult to deal with the enormity and complexity of these symbiont communities, especially given the limitations of approaches that depend on culturing bacteria, but recent advances in 'next-generation' sequencing methods and in bioinformatic distillation of the resulting data have rendered this area of investigation more accessible.

Mammalian fetuses are sterile; the neonate acquires its microbiota during and shortly after delivery. Once established, the repertoire of symbionts can fluctuate with alterations in the host diet or physiology; yet, it is stable enough over time that host kinship relations in microbial community compositions can be discerned (40, 41). The relative importance of

host environment versus genetics in shaping symbiont communities is still being debated (36). Certainly, phylogenetic influences can be detected, each mammalian species appearing to carry a 'core microbiome' at the gene level (41, 42). Yet, monozygotic and dizygotic twin pairs show a similar degree of variability in gut microbial communities (41). In any case, microbiota composition reflects eons of host–symbiont co-evolution (39, 40). These observations raise some interesting questions for immunologists to ponder: if an adult mammal harbors an order of magnitude more bacterial cells than there are somatic and germline cells in the entire body, is the microbiota to be considered 'self' or 'non-self'? Is it to be thought of as an environmental factor (because it responds to surrounding conditions) or an epigenetic factor (because it passes from generation to generation)?

The human and murine gut microbiota are surprisingly similar (36). It has been estimated that up to approximately 1000 different microbial species from approximately 10 different divisions colonize their intestinal tracts; but in both cases, just two bacterial divisions, the *Bacteroidetes* and *Firmicutes*, and one member of the *Archaea* dominate, together accounting for approximately 98% of 16S rRNA sequences obtained from the gut. The number and identity of symbiont communities vary along the length of the intestinal tract, in a proximal to distal gradient of abundance (small intestine < cecum < colon), and across the three dimensions of the lumen and mucous layers. The total number of genes borne by the gut microbiome has been estimated to exceed by more than a 100-fold that of the human genome. The products of these microbial genes are critical to the host, for example in digestion, production of nutrients, detoxification, defenses against pathogens, gut motility, angiogenesis and immunomodulation. A fascinating example is *Bacteroides plebius*, which, in the gut of Japanese but not North American individuals, expresses a porphyranase enzyme crucial for the digestion of seaweed, an element of the daily Japanese diet (43). The amazing potency – and disease relevance – of the intestinal microbiota was highlighted in recent reports that obesity (44), the metabolic syndrome (45), or ulcerative colitis (46) could be communicated to a normal mouse by gavage of fecal material from a diseased mouse.

Gut symbionts and the immune system

The microbiota, notably the intestinal microbiota, and the immune system are closely linked, each influencing and being influenced by the other (reviewed in 47). The incomplete state of the immune system in neonatal and in adult germ-free (GF) individuals argues that symbiont microbes drive correct maturation. These deficiencies include (but are not limited to) defective T, B, and innate cell compartments in gut-associated lymphoid tissue, fewer peripheral $CD4^+$ T cells, a systemic tilt to the Th2 phenotype, and reduced complements of IgG and IgA antibodies, all of which are reversed shortly after microbial colonization. These types of influences have been recognized for decades. Ground-breaking have been a growing number of examples of gut-residing bacteria, sometimes even a single species, driving the emergence, and/or stability of particular $CD4^+$ T cell subsets.

An early example was the finding that a defined set of bacteria promoted the development of a robust Th17 compartment in the small intestinal (SI) *lamina propria* (LP) of mice (48). Interestingly, individuals housed in certain specific-pathogen-free (SPF) facilities (e.g., Jackson Laboratory) but not others (e.g., Taconic Farms) lacked this element of the microbiota and had a defective SI-LP Th17 compartment. A contemporary study reported a similar influence of gut microbiota on the smaller population of Th17 cells residing in the large intestinal (LI) LP (49). Later, it was determined that a single microbial species, segmented-filamentous bacteria (SFB), could drive Th17 cell accumulation in the SI-LP (50, 51). SFB are Gram-positive, spore-forming, obligate anaerobes that have not yet been cultured *in vitro* (reviewed in 52). They colonize the gut at weaning, when they adhere tightly to ileal epithelial cells, particularly around the Peyer's patches. Sequencing of the genome of SFB derived from monoclonized mice was recently completed, identifying its closest relatives to be members of the genus *Clostridium* (53–55).

The genome sequence revealed SFBs to encode four types of flagellin, encouraging speculation that they may promote SI-LP Th17 cell differentiation through a Toll-like receptor (TLR)-dependent pathway; however, it was previously reported that the Th17 compartments of both the SI-LP and LI-LP do not depend on TLR signaling (48, 49). Instead, it has been proposed that SFBs induce secretion of serum amylase-A by intestinal epithelial cells, which, in turn, programs intestinal DCs to promote Th17 cell differentiation (50).

A second example of a defined set of bacteria promoting the differentiation and/or maintenance of a particular $CD4^+$ T-cell subset is the recent report that a mix of 46 strains of *Clostidium* drove the accumulation of a robust $Foxp3^+CD4^+$ Treg compartment in the mouse colon (56). Interestingly, the Treg population in the SI-LP (at least its numbers) was not dependent on the microbiota. Later (57), it was reported that colonic Tregs display a repertoire of TCRs distinct from that of Tregs at other sites, suggesting that local antigens might shape the colonic compartment. Indeed, it could be shown that such

antigens were derived from gut-resident microbes, prompting the proposal that microbial antigens somehow divert naïve T cells with corresponding specificities into the Treg lineage. This scenario is consistent with the finding that *Clostridium* species could induce intestinal epithelial cells to release active transforming growth factor-β and other mediators (e.g., indoleamine 2,3-dioxygenase) (56). Once again, signaling though TLRs did not seem to play a role in this microbial influence on the immune system.

It is very difficult, of course, to demonstrate such effects in humans, but it is known that human intestinal symbionts can promote specific $CD4^+$ T-cell compartments in mice. The best-characterized example is *B. fragilis*. This microbe and its product, polysaccharide A (PSA), can attenuate mouse models of colitis and experimental autoimmune encephalomyelitis (EAE), mobilizing mechanisms ranging from reduction of Th1 cells, induction of IL-10-producing $CD4^+$ T cells, diminishment of the Th17 compartment, and augmentation of Treg activity (58–61). One possibility to explain this somewhat disconcerting pleiotropism is that different processes come into play according to the dose, route or modality (*B. fragilis* versus PSA) of administration, or in different inflammatory contexts. A perhaps more satisfying explanation is that Tregs are the lead players, secondarily dampening Th17 or Th1 or antibody effector responses, depending on the particular immune setting. This interpretation would be consistent with the recent suggestion that PSA can signal Tregs directly through TLR-2, which in turn can restrain Th17 cells (61).

Gut symbionts and autoimmunity

Given the aforementioned associations between microbial and immune-system cells, it is not surprising that the microbiota has been tied to immune pathologies, notably allergies and autoimmune/inflammatory diseases (28, 29, 62). Comparisons of autoimmune disease development in different rodent models housed under GF versus SPF or conventional conditions have shown the full range of responses to loss of symbiont microbes: disease amelioration (63–65), no significant effect (66), or disease exacerbation (67). On first thought, it may seem perplexing that the microbiota can have opposing effects on the manifestations of autoimmune disease – even more so when there are divergent outcomes with models of presumably the same inflammatory disorder, e.g., arthritis (63, 64, 67), or even with the same model in the hands of different investigators, e.g., NOD mice (68, 69). More precise knowledge of the colonizing microbiota at different animal facilities and more profound understanding of the heterogeneity of the pathogenetic mechanisms underlying the various models should resolve many of these 'discrepancies'.

Localized links between the gut microbiota and inflammatory bowel disease or between skin microbial communities and psoriasis (and other inflammatory disorders of the skin) are easy to envisage. More intriguing is the evidence of specific influences of the microbiota on autoimmune manifestations at distal sites that is beginning to emerge. As described above, mice housed under GF conditions have few Th17 cells, in particular at their primary site of accumulation, the SI-LP; colonization of GF mice with the intestinal microbiota reflected in feces induced a robust Th17 compartment in less than a week (48). So did colonization with a single microbial species, SFB (50, 51). The implication of Th17 cells and/or IL-17 in a number of autoimmune diseases encouraged explorations of the influence of SFB in rodent models. Arthritis was greatly attenuated in the K/B × N model when housed GF (*vis-à-vis* SPF); robust disease was restored in less than 2 weeks after introduction of GF mice into an SPF facility or subsequent to SFB monocolonization (64). The pathogenic scenario was documented to be: SFB colonization → development of an SI-LP Th17 compartment → appearance of Th17 cells in the spleen, likely via migration from the gut → generation of arthritogenic B cells and autoantibodies in the spleen, promoted by a direct impact of IL-17A on B cells → autoantibody deposition in the joints. In the K/B × N model, the autoantibody deposition is a pivotal event, inducing arthritis by well-established mechanisms entailing the mobilization of inflammatory cells (neutrophils, MFs) and cytokines (e.g., IL-1) (70). This scenario is consistent with the observation that treatment of K/B × N mice with anti-IL-17 monoclonal antibody blocked the production of autoantibodies and the consequent development of arthritis (64). Next, parallel experiments were performed on an EAE model of multiple sclerosis. EAE was reduced in GF mice, associated with a reduction in Th17 cells (also fewer Th1 and more Treg cells); monocolonization with SFB induced EAE. Disease induction reflected an increased Th17 compartment in both the gut and spinal cord. Thus, a single gut-resident microbe can impact autoimmune manifestations distally in both joints and the central nervous system.

Of course, SFB is not the only culprit. For example, arthritis in SKG mice, a model that highlights the role of self-reactive T cells that escaped clonal deletion in the thymus (71), was more severe in a colony housed under conventional than under SPF conditions (72). Disease exacerbation in the less clean facility was attributed to fungal colonization, which, through a β-glucan/Dectin-1 interaction, induced arthritogenic Th17

cells in a complement-dependent manner (72, 73). Different again, arthritis in the *il-1rn*$^{-/-}$ model could be provoked by monocolonization of GF mice with *Lactobacillus bifidus*, through a TLR-4/IL-1/Th17 axis (63).

What about T1D?

The impact of the microbiota on T1D

It is often stated that the cleaner the NOD colony, the higher the diabetes incidence. Some data support this contention (68, 74), but other results do not, demonstrating, in particular, that GF and SPF NOD colonies show a similar course and penetrance of autoimmune diabetes (69, 75, 76).

Evidence that specific elements of the microbiota impact the NOD disease is beginning to emerge. Wen *et al.* (75) reported that MyD88-deficient NOD mice housed in an SPF facility were protected from diabetes development, but transfer to GF housing led to robust disease, like that of wildtype-NOD mice kept under both conditions. Colonization of the GF mutant line with a defined set of microbes normally resident in the human intestinal tract (known as the altered Schaedler flora) attenuated disease, as did exposure to microbiota of SPF-housed mutants. 16s rRNA sequencing of cecal DNA showed the SPF-housed MyD88-deficient NOD mice to have an altered microbiota, e.g. a lower *Firmicutes/Bacteroidetes* ratio. Nonetheless, it will be important to distinguish the specific impact of the MyD88 deficiency from that of the general health status on diabetes development.

More recently, single bacterial species have been associated with protection from diabetes in the NOD model. King and Sarvetnick (69) cited *Bacillus cereus*, providing little additional information. Kriegel *et al.* (68) reported that SFB was correlated with diabetes protection. They found that individuals housed in the same NOD colony varied in their SFB status and that this was clearly reflected in their relative susceptibility to disease: while almost all females that were free of this microbe developed T1D, only about 15% that carried it got diabetes. In contrast, both SFB-positive and -negative individuals exhibited robust insulitis. Proof of a role for SFB in disease protection, and whether it is a direct one or is mediated through co-symbionts, must await monocolonization and co-housing experiments. In the meantime, the only difference found in the immune systems of SFB-positive and -negative female NOD mice was a greatly reduced SI-LP Th17 population in the latter. Also awaiting further study is whether and which microbes were responsible for the low diabetes incidence in male NOD mice independent of SFB status.

These observations, in conjunction with the arthritis study mentioned above (64), raise an interesting question: how does SFB promote autoimmune disease in one context and dampen it in another? This divergent effect is likely a reflection of the fact that not all autoimmune disorders have the same mechanisms of initiation, propagation, and regulation. Just as it was too naive in the 1990s to think that manifestations of autoimmunity always reflect a Th1/Th2 imbalance, it is an over-simplification today to expect that they universally signal dysregulation of the Th17/Treg balance. Considering, for example, the models discussed above: murine arthritis models typically have a strong Th17 dependency; there is still active debate over the relative importance of Th1 and Th17 cells in EAE; and there is little, and contradictory, support for a critical role for Th17 (over Th1) cells in NOD diabetes (68). Th subsets are known to cross-inhibit, so it follows that a Th17-inducing microbe (like SFB) could potentially inhibit a Th1-dependent disease (like NOD diabetes). Alternatively, another SFB activity might show a differential impact in different autoimmune contexts. For example, SFB induces IL-22 expression as well (50, 68), and this cytokine's ability to repair intestinal epithelium might counter breaches of the intestinal barrier thought to promote T1D (77, 78), but not known to influence arthritis or EAE.

As we stand today, it seems clear that the gut microbiota can have an important impact on the penetrance and progression of autoimmune diabetes in murine models. But, we are at the threshold of understanding which specific microbes are responsible and precisely how they exert their influences. It might be instructive at this point to return to the framework of T1D pathogenesis discussed above and illustrated in *Fig.* 1 and consider potential points of the microbiota's impact (*Fig.* 2). As concerns checkpoint 0, establishment of a self-reactive lymphocyte repertoire, it is theoretically possible that symbiont microbes (and proteins or peptides derived from them) help shape positive and negative selection of T and B cells. While an example of such a scenario is yet to be provided, we are seeing more and more cases of peripheral DCs trafficking to the thymus, and their cargoes imposing constraints on T-cell selection (reviewed in 79). Then, why not gut-derived DCs delivering microbe-derived antigens? As for the initiation of insulitis, checkpoint 1, it is known that intestinal symbionts regulate the permeability of the intestinal epithelial barrier, and compromised barrier permeability has been repeatedly associated with T1D in patients and both the NOD mouse and BB rat models (77, 78, 80–82). Indeed, such a defect may underlie the frequent association of T1D and celiac disease (83). This axis of influence may be enhanced by a preferential trafficking route from the gut to the pancreatic lymph nodes, where T cells are effectively activated by

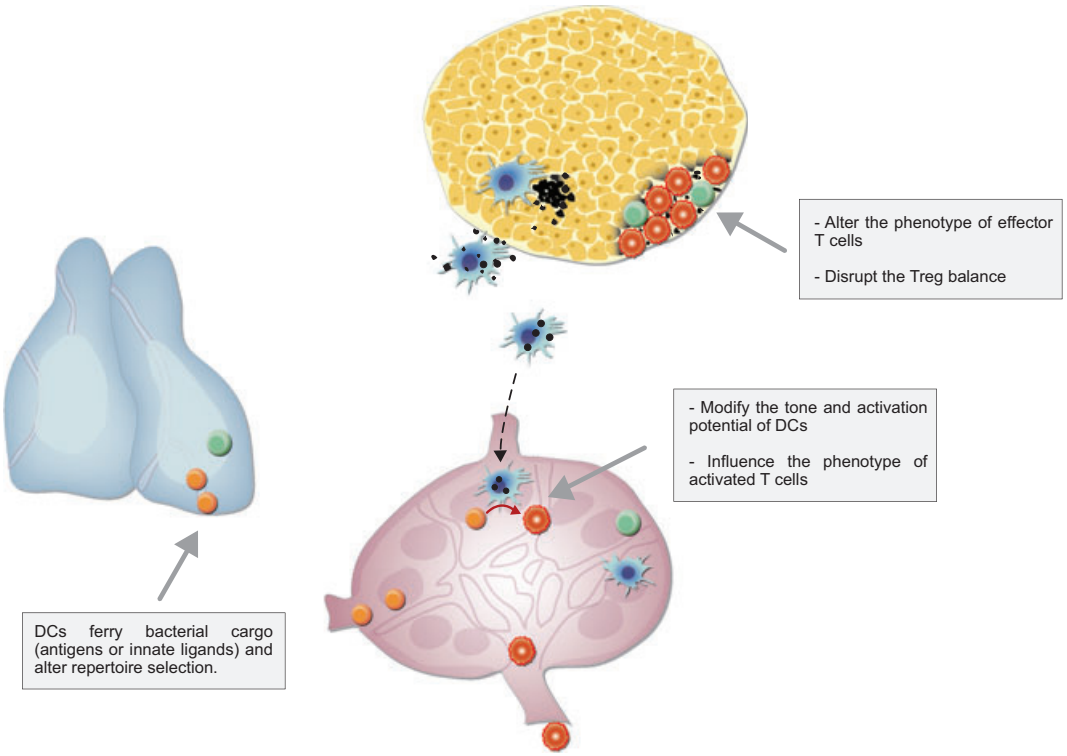

Fig. 2. Influence of the microbiome on diabetes checkpoints.

antigens derived from the gastrointestinal tract and peritoneum (84). Lastly, in relation to both checkpoint 1 and checkpoint 2, the conversion of insulitis to diabetes, we have already detailed a growing body of evidence that specific microbes can drive the accumulation of particular $CD4^+$ T-cell compartments in the intestine, and that effects of these populations can be detected systemically. Th17 (as well as other Th subsets) and Treg cells can dictate the flavor of the T-cell response upon initial encounter of β-cell derived antigens in the pancreatic lymph nodes, and can regulate lymphocyte activities within the evolving islet lesion.

On a related note, might long-recognized but little understood MHC or HLA associations with T1D at least in part reflect influences on symbiont microbe colonization? Do MHC-Eα and HLA-DQ2 molecules protect from diabetes through an influence on one or more elements of the microbiota? There has, for example, been a report that the MHC/HLA-like molecule, CD1d, could regulate the composition of bacterial communities in the mouse gut (85).

Beyond T1D models to patients

Given the daunting complexity of the microbiota and microbiome, coupled with the complicated nature of and our current ignorance about the pathogenesis of T1D, we need to rely primarily on animal models to probe the mechanisms of microbial impact on development of autoimmune diabetes. Yet, translation to the human context is the ultimate goal. While intriguing preliminary data have been reported, arguing for a perturbation of microbial communities in young children with anti-pancreas autoantibodies that are genetically at risk of contracting T1D (86), this line of work is in its infancy.

Elucidating these issues in humans will be difficult and complex. Microbiome-wide association studies are no doubt in progress in several laboratories, but these endeavors are likely to be subject to some of the same weaknesses as are genome-wide association studies – even more so, given that, while an individual's genome is constant, the microbiome fluctuates with time, diet changes, drug treatment, etc. It may be helpful to develop culture systems that capture the interactions between microbial, immune-system, and intestinal cells, but such systems are likely to be challenging, requiring the correct three-dimensional structure, optimally under anaerobic conditions. Stem-cell technology may help (87). No doubt, we will continue to rely on rodent models, in particular humanized-mouse models, to elucidate critical principles. Murine and human immune systems are much more similar than they are different; the species' microbiota share dominant groupings, although there are many divergences at the lower taxonomy levels (36). Encouragingly, human-gut-derived symbionts like

B. fragilis have been reported to colonize the mouse intestine, impact the immune system, and modulate autoimmune and inflammatory diseases (58, 59).

Regardless of the impediments, studies on the microbiota and microbiome open new vistas on human autoimmune diabetes. There may or may not prove to be associations between T1D and particular symbionts; their identification may or may not yield novel approaches to prevention or treatment – entailing administration of probiotics or drugs. But even independent of such associations, the microbiome promises to be an amazingly rich source of novel immunomodulatory molecules. We should exploit the fact that it has co-evolved with its host for eons, developing a multitude of strategies to tame the immune system.

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