Protective major histocompatibility complex allele prevents type 1 diabetes by shaping the intestinal microbiota early in ontogeny

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Certain MHC-II or HLA-D alleles dominantly protect from particular autoimmune diseases. For example, expression of the MHC-II Eα:Eβ complex potently protects nonobese diabetic (NOD) mice, which normally lack this isotype, from spontaneous development of type 1 diabetes. However, the underlying mechanisms remain debated. We investigated MHC-II–mediated protection from type 1 diabetes using a previously reported NOD mouse line expressing an Eα transgene and, thereby, the Eα:Eβ complex. Eα16/NOD females vertically protected their NOD offspring from diabetes and insulitis, an effect that was dependent on the intestinal microbiota; moreover, they developed autoimmunity when treated with certain antibiotics or raised in a germ-free environment. Genomic and proteomic analyses revealed NOD and Eα16/NOD mice to host mild but significant differences in the intestinal microbiotas during a critical early window of ontogeny, and transfer of cecal contents from the latter to the former suppressed insulitis. Thus, protection from autoimmunity afforded by particular MHC/HLA alleles can operate via intestinal microbes, highlighting potentially important societal implications of treating infants, or even just their pregnant mothers, with antibiotics.

microbiome | type 1 diabetes | neonatal | autoimmune disease | NOD mice

Type 1 diabetes (T1D) is an autoimmune disease characterized by T cell-provoked destruction of the insulin-producing β-cells of the pancreatic islets of Langerhans. Development of autoimmune diabetes is regulated by multiple genetic polymorphisms and largely unknown environmental factors. This and many other autoimmune diseases have their strongest genetic association with the HLA-D locus (1). Certain HLA-D haplotypes, such as DRB1*0401-DQB1*0302 and DRB1*0301-DQB1*0201, confer elevated risk for T1D; others, notably DRB1*1501-DQB1*0602, promote dominant protection (estimated to be as high as 97%) (2). Interestingly, an HLA allele that protects from one autoimmune disease can promote another.

The nonobese diabetic (NOD) mouse strain is the most widely studied animal model of autoimmune diabetes. All NOD mice develop a leukocytic infiltrate in their pancreatic islets, termed insulitis, around 3–4 wk of age, and a fraction of them progress to overt diabetes starting at about 12–14 wk, depending on the particular colony. Similar to the disease in humans, diabetes in NOD mice is a T cell-dependent, polygenic disorder that is modified by environmental factors. Parallel to the situation in humans, the MHC locus is by far the dominant genetic determinant in mice (1). The NOD strain expresses an unusual MHC-II A complex, termed A^{g7}, and does not express an E complex due to deletion of the *Eα* promoter (3). Remarkably, NOD mice genetically modified to express the Eα molecule in the appropriate cells, are completely protected from T1D and are either entirely or nearly devoid of insulitis (4–6).

Thus far, there is no clear consensus on the mechanism of E-mediated protection from T1D. An early model proposed that E complex expression leads to clonal deletion or anergizing of autoreactive T cells (7). But such a mechanism was rendered unlikely when clonal deletion in E-expressing NOD mice could be dissociated from protection from insulitis (5), and when E expression in the thymus was found to be neither necessary nor sufficient for protection (8). In addition, E^+ NOD mice have islet cell-reactive T cells that can transfer disease to T cell-deficient NOD mice, arguing that they have a diabetes-competent T lymphocyte repertoire (9, 10). A second model argued for altered autoantigen presentation in E-expressing NOD mice: the E complex would outcompete A^{g7} for limited pathogenic peptides. This mechanism also proved unlikely because E^+ and E^- antigen-presenting cells (APCs) from NOD mice similarly present peptide to and prime autoreactive T cells in vitro and in vivo (11, 12). A third proposed mechanism, that E complex expression alters the cytokine skewing of CD4$^+$ T effector cells or promotes the generation of Foxp3$^+$ T regulatory (T$_{reg}$) cells, has been supported by data from some studies (5) but refuted by results from others (13).

There is a critical role for nongenetic (e.g., environmental) factors in the development of T1D in both humans and mice. The rapid rise of T1D incidence over the past few decades argues for an important nongenetic component to the pathogenesis of

Significance

This report brings a new perspective on the decades-old question of how MHC and HLA complexes can potently protect against a variety of autoimmune diseases, including type 1 diabetes. We demonstrated that protection by the MHC-II Eα:Eβ complex operated via modulation of the composition of the intestinal microbiota during a critical early window of ontogeny, associated with modification of the local immune system. These findings prompt a model of HLA/MHC-mediated protection from autoimmunity, and raise the question of whether disease-protective alleles in other human autoimmune diseases or models thereof might operate by a similar mechanism. They also argue that treating infants and pregnant mothers with antibiotics should be minimized.

T1D (14), as do epidemiologic studies demonstrating that monozygotic twins have less than 50% concordance for T1D (15). Recent results have highlighted a role for the intestinal microbiota in promoting or protecting from several autoimmune diseases, including this one (16, 17). Since a few reports have suggested that MHC/HLA complexes can influence microbial colonization of the gut (18, 19), although others have appeared to disagree (20), we hypothesized that E-mediated protection from NOD autoimmunity might be an indirect effect, channeled through influences on the intestinal microbiota. Using multiple experimental approaches, we demonstrate this hypothesis to be true.

Results

Eα16/NOD Dams Transmitted Protection from Insulitis and T1D Vertically to Their NOD Progeny.

Since the maternal and neonatal environments can influence diabetes development in both NOD mice (21) and humans (22), we began by investigating whether protection from diabetes could be vertically transferred from an Eα16/NOD dam to her NOD progeny. Diabetes incidence was compared in cohorts of female NOD mice born to either NOD dams mated to Eα16/NOD sires or to the reciprocal combination. NOD progeny of Eα16/NOD dams had a significantly reduced incidence (39% vs. 65%) and delayed onset (21 vs. 14 wk of age) of diabetes *vis á vis* NOD progeny of NOD dams (Fig. 1A). Since multiple checkpoints are involved in the development of T1D, we investigated whether insulitis was also affected, and found that the NOD progeny of Eα16/NOD dams had significantly reduced insulitis as well (Fig. 1 B and C).

Genetic imprinting, maternal antibody transfer, and microbiome colonization are all possible mechanisms for such vertically transmitted protection. Since the microbiota can protect NOD mice from disease development in certain circumstances (17), we tested whether maternal microbes were responsible for E-mediated vertical suppression of insulitis by giving vancomycin in the drinking water of Eα16/NOD mothers during their last 7–10 d of pregnancy. This treatment resulted in an insulitis frequency in NOD progeny of Eα16/NOD dams that was significantly higher than that of offspring from their untreated counterparts, and was indistinguishable from that of progeny from standard NOD dams (Fig. 1D). Thus, E-mediated protection from autoimmunity was, at least to a degree, vertically transmitted to NOD progeny and was microbiota-dependent.

Treatment with Certain Antibiotics Induced Insulitis and Altered the Intestinal Microbiome in Eα16/NOD Mice.

This conclusion implied that disruption or loss of the microbiota in Eα16/NOD mice might induce autoimmunity. As a first approach to evaluating this possibility, we gave female Eα16/NOD mice antibiotics in the drinking water, choosing vancomycin, metronidazole, neomycin, and ampicillin to survey a broad range of microbe sensitivities. Oral treatment from 3 to 6 wk of age with vancomycin or metronidazole, but not with neomycin or ampicillin, induced insulitis in a fraction of the Eα16/NOD mice (Fig. 2A); when it appeared, islet infiltration could be quite severe (Fig. 2B). While the fraction of Eα16/NOD mice showing insulitis under these conditions was quite low, this result contrasted with the extremely rare and mild insulitis observed with their untreated counterparts. Nonetheless, we sought to further optimize the treatment protocol. The effectiveness of vancomycin treatment showed a clear age-dependence. Giving this antibiotic either from the last 7–10 d of pregnancy until 3 wk after birth (optimally) or from 3 to 6 wk of age induced insulitis in Eα16/NOD mice, but administering it from 6 to 10 wk of age did not (Fig. 2C).

Since oral vancomycin is not systemically absorbed, it appeared that disruption specifically of the intestinal microbiota might induce autoimmunity in a fraction of Eα16/NOD mice. We addressed this possibility by characterizing the fecal microbiome of vancomycin-treated pregnant dams, their progeny, and control mice unexposed
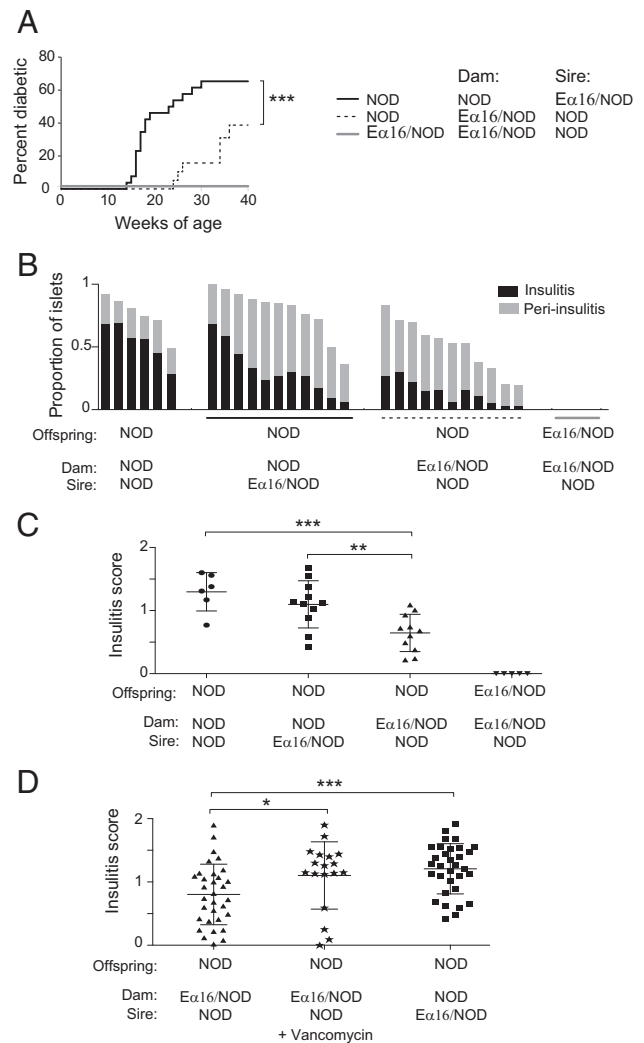

Fig. 1. Eα16/NOD dams transmitted protection from insulitis and T1D vertically to their NOD progeny. (A) Diabetes incidence in a cohort of NOD mice born to NOD dams (n = 34), NOD mice born to Eα16/NOD dams (n = 24), and Eα16/NOD mice (n = 20). ***P = 0.0004 (Gehan–Breslow–Wilcoxon test). (B) Proportion of islets with insulitis or peri-insulitis at 10 wk of age. NOD mice born to NOD dams mated with NOD sires, NOD mice born to NOD dams mated with Eα16/NOD sires, NOD mice born to Eα16/NOD dams mated to NOD sires, and Eα16/NOD mice. (C) Composite insulitis score for each mouse in B. ***P = 0.001, **P = 0.005 (Mann–Whitney test). (D) Composite insulitis scores for vancomycin-treated Eα16/NOD dams that received oral vancomycin during the last 7–10 d of pregnancy. ***P = 0.0005, *P = 0.02 (Mann–Whitney test).

to vancomycin by sequencing the V4 region of the 16S ribosomal RNA (rRNA) gene. As expected, oral administration of vancomycin changed the intestinal microbiome (reflected in feces) of the Eα16/NOD pregnant dams; the altered maternal microbiome was transmitted to their progeny, persisting through 10 wk of age (Fig. 2D). Principal coordinates analysis (PCoA) of unweighted UniFrac distances, with each dot representing the microbiome of an individual mouse, indicated that mice directly or indirectly exposed to vancomycin clustered separately from those that received no antibiotic (Fig. 2E, Upper). Most interesting, the altered microbiota was transmitted from vancomycin-treated Eα16/NOD dams to pups that had not been directly exposed to vancomycin. Consistent with this observation and the expected normalization of microbial flora over time, the maternal microbiomes clustered closer to those of their 3-wk-old than their 10-wk-old progeny (Fig. 2E, Lower).
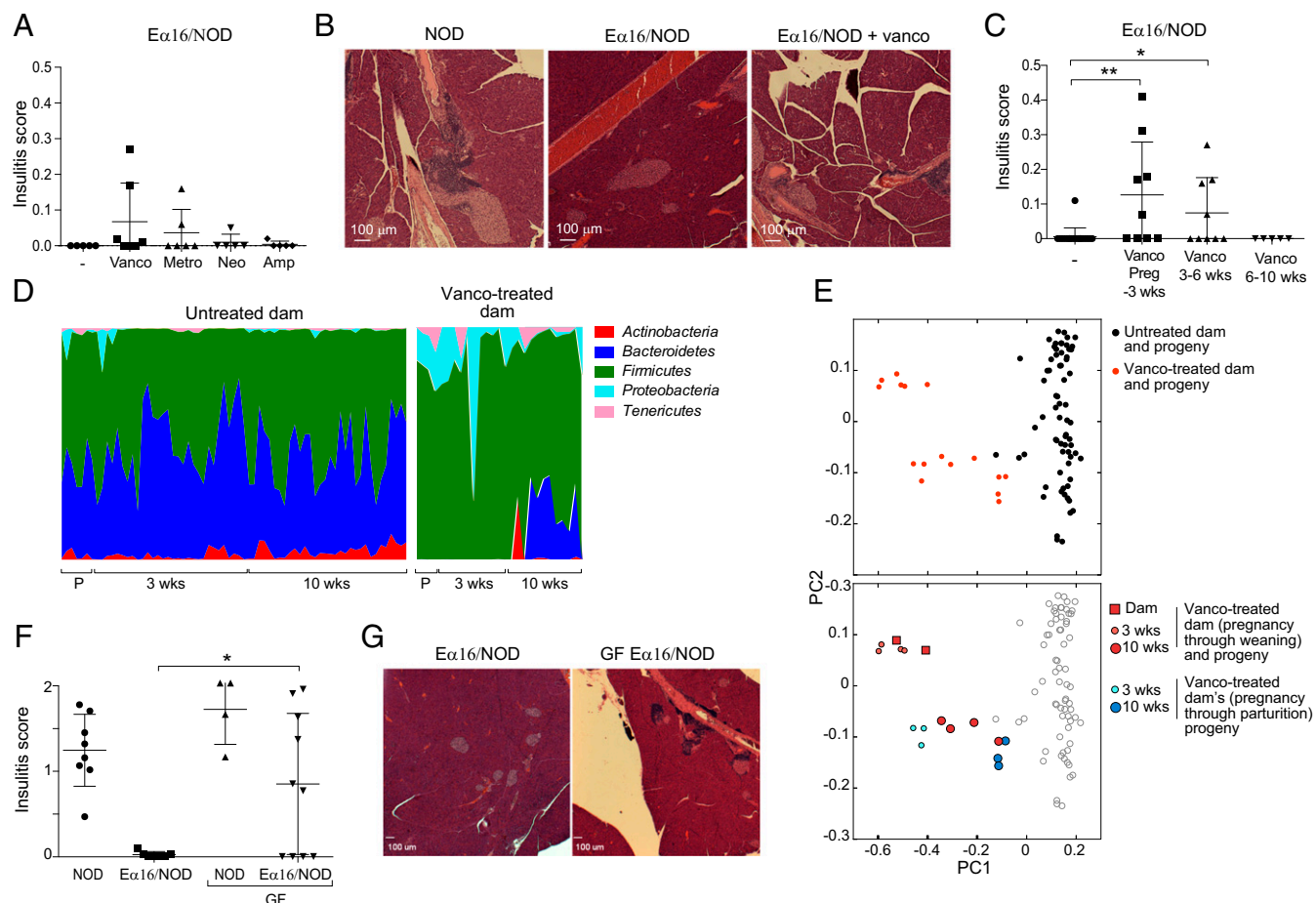
Fig. 2. Antibiotic treatment induced insulitis and altered the intestinal microbiome in Eα16/NOD mice. (*A*) Insulitis composite score in Eα16/NOD mice treated with oral antibiotics provided in their drinking water from 3 to 6 wk of age. (*B*) Pancreas histology of 10-wk-old NOD mice (*Left*), Eα16/NOD mice (*Center*) or Eα16/NOD mice treated with vancomycin from 3 to 6 wk of age (*Right*). (*C*) Composite insulitis score of Eα16/NOD mice treated with vancomycin over different time periods, which include the last 7–10 d of pregnancy through weaning of the pups at 3 wk of age, 3–6 wk of age, or 6–10 wk of age. **$P = 0.002$, *$P = 0.03$ (Mann–Whitney test). (*D*) Phylum-level representation of 16S rRNA fecal microbiome from control or vancomycin-treated parents and their progeny at 3 and 10 wk of age. P, parents; 3 wk, 3-wk-old progeny; 10 wk, 10-wk-old progeny. (*E*) PCoA of unweighted UniFrac distances calculated from 16S rRNA gene sequencing of these fecal samples. (*F*) Insulitis scores from NOD, Eα16/NOD, and GF Eα16/NOD and NOD mice at 16–20 wk of age. *$P = 0.02$ (unpaired *t* test). (*G*) Pancreas histology from Eα16/NOD and GF Eα16/NOD mice.

Furthermore, we generated germ-free (GF) NOD and Eα16/NOD mice, and compared their degrees of insulitis. Sixty percent of GF Eα16/NOD mice showed substantial insulitis, with a severity similar to that of both their GF NOD littermates and standard NOD mice (Fig. 2 *F* and *G*). In short then, data from both the antibiotic-treatment and GF-housing experiments argued that microbes were critical elements of E-mediated protection from NOD diabetes. While the effects were not completely penetrant, it is important to keep in mind that only a few untreated Eα16/NOD individuals from our colony, of the hundreds examined over the past 25 y, exhibited any insulitis (5).

Eα16/NOD Mice Hosted a Distinct Intestinal Microbiome Early in Ontogeny. Islet autoimmunity begins when diabetogenic β-cell–derived self-antigens first appear in the pancreas-draining lymph nodes (PLNs) at days 15–18 in NOD mice (23), which also corresponds to the time when lymphatic connections from the gastrointestinal tract to the PLNs develop (24). Therefore, we tested whether expression of the E molecule impacted the microbiome of 18-d-old mice via 16S rRNA gene sequencing of cecal contents. To avoid detecting spurious associations between E-molecule expression and microbial taxa due to maternal or cage effects, we compared only cohoused NOD ($n = 25$) and Eα16/NOD ($n = 25$) littermates. The α-diversity of the cecal contents microbiome was

significantly higher in Eα16/NOD than in NOD mice, and β-diversity was significantly lower, indicating that the microbiomes of the two types of mice were distinct (Fig. 3 *A* and *B*). Taxonomic comparisons by generalized linear mixed-effects modeling revealed a higher representation of microbes from the order Clostridiales [false-discovery rate (FDR) -corrected $P = 1.2 \times 10^{-3}$] and a lower proportion of the genus Blautia (FDR-corrected $P = 7.2 \times 10^{-6}$) in the microbiomes of Eα16/NOD mice (Fig. 3C, Fig. S1, and Table S1).

Since certain tissue-associated microbes may engender specific immune responses, we next investigated whether cecal tissue-associated microbes were differently represented between Eα16/NOD and littermate NOD mice. Consistent with the findings in cecal-content microbiomes, a higher representation of microbes from the order Clostridiales (FDR-corrected $P = 1.4 \times 10^{-7}$) and a lower proportion of the genus Blautia (FDR-corrected $P = 2.7 \times 10^{-14}$) were present in the cecal tissue microbiomes of Eα16/NOD mice (Fig. 3C, Fig. S1, and Table S1).

The microbiota differences were not uniform in all mice from different cages (Fig. S1), suggesting redundancy in the range of microbes affected by the E molecule. Therefore, we sought to identify combinations of operational taxonomic units (OTUs) affected by E (and interfering with diabetes) by constructing Random Forest classifiers of E or N genotypes based on the microbiome profiles.
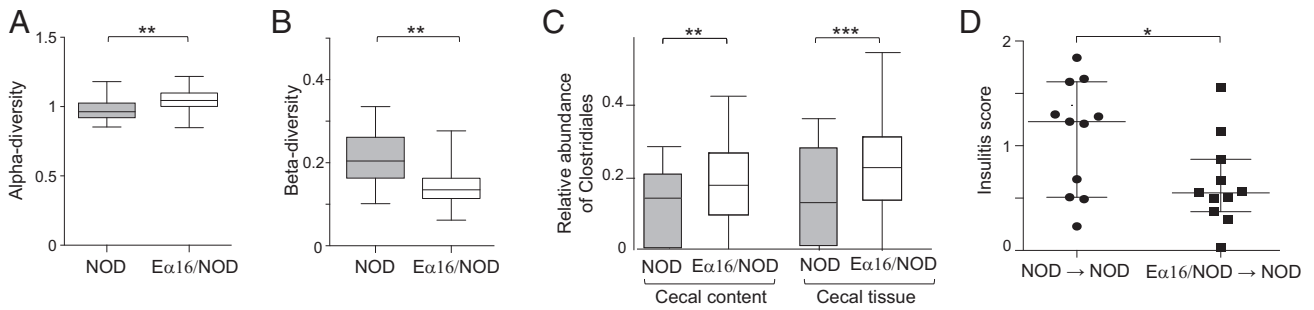
Fig. 3. NOD and Eα16/NOD mice host distinctive intestinal microbiomes. (*A*) Boxplots of cecal microbiome α-diversity (PD whole tree) normalized as the ratio of each sample's α-diversity to its cage mean. $n = 25$ Eα16/NOD and $n = 25$ NOD mice (**$P = 0.004$). (*B*) Boxplots of cecal microbiome β-diversity (weighted UniFrac distance) (**$P = 0.0098$). Unpaired *t* test with 10,000 Monte-Carlo simulations. (*C*) Boxplots of the relative abundance of the order Clostridiales in cecal contents and tissue. **$P < 0.01$, ***$P < 0.001$. (*D*) Cecal contents from NOD or Eα16/NOD donors were gavaged to NOD pups twice weekly from 2 to 5 wk of age. Insulitis was assessed at 10 wk of age. *$P = 0.04$ (unpaired *t* test).

While success rates of 80–87% could be obtained, these proved not significantly different from chance by permutation analysis.

We also exploited a metaproteomic approach to investigate microbial protein expression in feces from littermate Eα16/NOD vs. NOD mice at day 18 and day 70 ($n = 24$ mice in total). Microbial proteomic data were aggregated according to both functional and taxonomic annotations (1,078 different functional-taxonomic features identified). α-Diversity was again significantly higher in Eα16/NOD individuals at day 18 and day 70. β-Diversity analysis revealed a strong cage/littermate effect at day 18, and clustering by mouse genotype at day 70 (Fig. S2).

Both the genomic and proteomic data argued that the Eα16/NOD and NOD microbiota were distinct during early life. The intestinal microbiomes of Eα16/NOD mice showed increased α-diversity, which has been associated with protection from T1D in genetically susceptible mice and humans (25, 26). To directly confirm this conclusion, we compared the ability of intestinal microbiota from Eα16/NOD vs. NOD donors to protect NOD recipients from insulitis. NOD mice gavaged twice weekly from 2 to 5 wk of age with Eα16/NOD cecal contents had a significantly reduced insulitis severity (median insulitis score of 0.55 vs. 1.23) compared with that of controls gavaged in parallel with NOD cecal contents (Fig. 3*D*).

Comparing the IgA-Bound Repertoire of Cecal Bacteria in NOD and Eα16/NOD Mice. To explore one possible mechanism for E-mediated, microbiota-dependent protection in the NOD model of T1D, we investigated the IgA response to gut bacteria in NOD and Eα16/NOD littermates born to NOD mothers, using the recently developed IgA-seq method (27), to interrogate the composition of microbes that are bound, or not, to IgA molecules in the intestinal contents. Eα16/NOD and NOD littermates did not differ in the flow cytometric frequency or mean fluorescence intensity of IgA-coated cecal bacteria (Fig. S3), contrary to previous data demonstrating fluctuations in the proportion of IgA$^+$ stool bacteria depending on the host MHC allele (19). In our IgA-sequencing data, direct comparisons between the genotypes revealed no OTUs that had significantly different IgA-coating index (ICI) values for either age group (Fig. 4 *A* and *B*). However, the LEfSe biomarker discovery tool (28) flagged a handful of OTUs that were overall differentially IgA-coated between NOD and their Eα16/NOD counterparts (Fig. 4 *C* and *D*), albeit with rather low scores. All four of the OTUs with higher representation in the Eα16/NOD IgA-coated microbiomes were from the order Clostridiales, while those with higher representations in NOD microbiomes were from the orders Clostridiales, Oceanospirillales, and Pseudomonales. When displayed on a per mouse basis and accounting for cage of origin, it appeared that the differential signals were scattered, with little uniformity, and were mostly due

to a few mice (Fig. S3). We again applied a Random Forest approach to identify an effect of E on IgA coating of combinations of microbes, but no groups of OTUs were identified whose ICI significantly distinguished (by permutation testing) the two genotypes.

Investigating the Intestinal Immune System in Eα16/NOD Mice. Finally, we explored whether the early-life microbiome alterations found in Eα16/NOD mice were associated with changes in the intestinal immune system. The innate and adaptive immune-system cell populations of the lamina propria of the large and small intestines, PLNs, Peyer's patches, cecal patch, and spleen
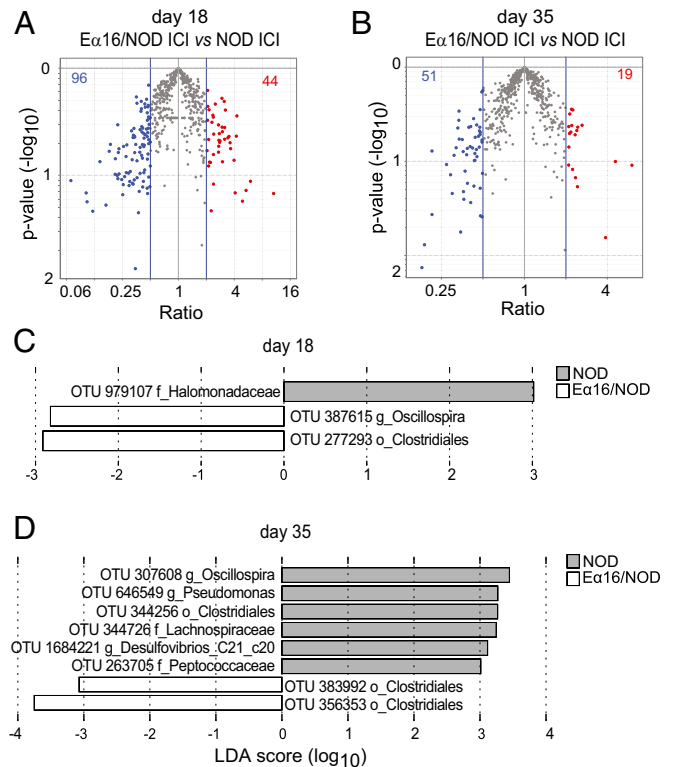

Fig. 4. IgA-bound repertoire of cecal bacteria is similar in NOD and Eα16/NOD mice. (*A* and *B*) Volcano plot comparing the ICI of the cecal microbiotas from NOD and Eα16/NOD mice at 18 and 35 d of age. (*C* and *D*) Comparison of the representation of specific IgA-coated taxa between the cecal microbiomes of 18- and 35-d-old NOD and Eα16/NOD mice.

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showed no robust differences in Eα16/NOD vs. NOD littermates, according to fraction and number of CD4$^+$ T-helper cells expressing IFN-γ, IL-17, IL-10, or IL-22, CD8$^+$ T cells, and myeloid cell populations, such as CD103-expressing cells. Since MHC-II expression on innate lymphoid cells (ILCs) is necessary to maintain homeostasis with commensal microbiota (29), we also compared the number and frequency of ILCs expressing the transcription factors Gata-3 or Rorγ, along with the number and frequency of ILC3 expressing IFN-γ, IL-17, or IL-22, and found no consistent differences (Fig. S4). However, we did observe an increased regulatory T_{reg} cell frequency in the cecal lamina propria of Eα16/NOD mice at day 18 (Fig. 5 *A* and *B*). In contrast, there were similar populations of T_{reg} in the small and large intestines, Peyer's patches, cecal patch, and the PLNs (Fig. 5*C*).

Discussion

An association between certain MHC and HLA class II alleles and protection from particular autoimmune diseases, notably T1D, was discovered decades ago; yet the mechanisms underlying this dominant protection have remained mysterious. Here we showed that commensal microbes drove E-mediated protection from autoimmune diabetes, and that expression of the E complex shaped the intestinal microbiome during a critical early window of ontogeny.

How might E complex expression shape the intestinal microbiota in young NOD mice? One possibility is that an additional restriction element, the E complex, could allow immune responses against additional microbial antigens, thereby shaping the developing microbiota. In particular, there could be an effect on the nascent IgA repertoire via presentation of additional microbial antigens, and IgAs are known, in turn, to shape the intestinal microbiota (19, 30). We tested the hypothesis that the type of MHC class II allele present would influence the affinity and specificity of the IgA produced in response to the intestinal microbiota of young Eα16/NOD vs. NOD mice. However, we saw no differences in the composition of IgA-bound bacteria. Nonetheless, IgA binding may alter microbial localization and function without detectable impacts on microbiota composition, as has been reported in some contexts (31).

Second, E expression might influence antimicrobial peptide (AMP) secretion from intestinal epithelial cells. Expression of the E complex on APCs, leading to more cognate interactions with CD4$^+$ T cells, could enhance APC activation, which in turn could trigger intestinal epithelial cells to secrete AMPs, known to impact intestinal microbiota localization and composition (32, 33). Third, E complex expression might promote host production of a microbe-specific metabolic substrate, conferring a selective advantage to specific microbes that can use this resource (34).

How does the Eα16/NOD intestinal microbiota prevent autoimmunity? A likely possibility is that the microbiome influences the development of the local intestinal immune system that, in turn, somehow prevents insulitis. Indeed, the intestinal microbiome changes in Eα16/NOD mice occurred at the time insulitis typically initiates (∼3 wk of age) (23). This timing corresponds to a wave of islet cell apoptosis (35) and establishment of a lymphatic connection between the intestinal immune system (24) and the PLNs, which is critical for the development of insulitis and T1D (36). The notion of an intestinal impact on the development of T1D is supported by studies in humans and mice, and is often referred to as the "leaky gut hypothesis" (37). In support of a role for the E complex working via such mechanisms, cell transfer experiments indicated that protection is mediated by the E-expressing macrophage or dendritic cell lineage (13), which is supported by genetic ablation studies showing a requirement for E-expression on the CD11c$^+$ but not CD19$^+$ cell lineage (12). Our observation of increased T_{reg} cell proportions in the cecum of Eα16/NOD mice could represent an effect of early-life microbial stimulation on CD11c$^+$ tolerogenic dendritic cells. Interestingly, Ooi et al. (38) have recently reported that the dominant protective effect of HLA-DR1 allele on development of Goodpasture's disease can be attributed to shaping of the self-epitope–specific T_{reg} repertoire.

Perhaps our study's most important message is a societal one, assuming a translation of our findings on the NOD model to human T1D patients. Antibiotic treatment of infants, or just their pregnant mothers, can potentially subvert ordinarily potent diabetes-protective genetic elements.

Materials and Methods

Mice. The generation of *Eα16*/NOD mice has previously been described (5). All mouse experiments were approved by the Institutional Animal Care and Use Committee of Harvard Medical School.

Diabetes and Insulitis Assessments. Diabetes and insulitis were assessed as previously described (39). Insulitis was scored by two independent readers who were blinded to the identity of the slides.

Gavage. NOD mice were gavaged biweekly starting at 2 wk of age using a 22-gauge straight oral gavage needle (VWR 20068-608) with either NOD or Eα16/NOD cecal contents.

Antibiotic Treatment. For antibiotic treatment, 1 g/L of ampicillin sodium salt (Sigma), 1 g/L of metronidazole (Acros Organics) plus the sweetener Equal 2.5 g/L, 0.5 g/L vancomycin hydrochloride (Acros Organics), or 1 g/L of neomycin (Fisher BioReagents) were used. Pregnant dams were provided with 0.5 g/L of vancomycin hydrochloride in their drinking water during the last 7–10 d pregnancy.

Sample Collection and DNA Isolation. Fresh fecal pellets, cecal contents, and cecal tissues were collected into sterile Eppendorf tubes under a laminar flow hood and stored at −80 °C until processing. Genomic DNA was isolated as previously described (39).

16S rRNA Gene Sequencing and Analysis. The 16S rRNA gene sequencing of the V4 variable region was performed at the Broad Institute or Biopolymers Facility at Harvard Medical School on the Illumina MiSeq platform using the protocol previously described (40). Sequences were processed and curated using QIIME v1.9.0, as pick_closed reference otus.spy (41). To control for maternal and cage effects, each sample's α-diversity value was normalized by dividing it against the mean α-diversity value calculated from all mice from its cage. This cage-normalized α-diversity value was then used for comparisons between NOD and Eα16/NOD mice. To compare β-diversity, weighted and unweighted UniFrac
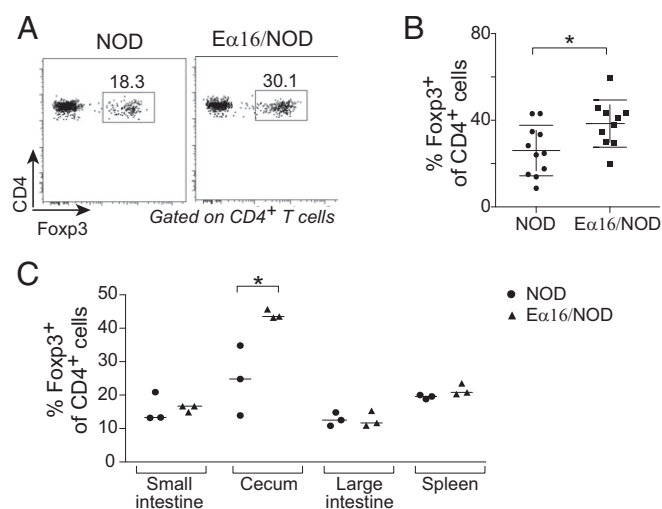
Fig. 5. Eα16/NOD mice had an enlarged representation of cecal T_{reg}. (*A*) Cells were isolated from littermates at day 18 and analyzed by flow cytometry. Representative plot of Foxp3$^+$CD4$^+$ cell population. Cells were gated as TCRβ$^+$CD19$^-$CD45$^+$. (*B*) Percentage of cecal lamina propria Foxp3$^+$CD4$^+$ T-cell from three independent experiments. (*C*) Percentage of Foxp3$^+$CD4$^+$ T cells. *P < 0.05.

distances were calculated between each pair of NOD mice and each pair of Eα16/NOD mice. To control for maternal and cage effects, UniFrac distances were calculated between cohoused littermate pairs.

Linear Mixed Effect Modeling. Data files from QIIME were analyzed in the R environment. Taxon differential abundance was calculated for the taxa that have greater than 1% relative abundance across all tested samples using generalized linear mixed-effects models with genotype and sample type as fixed effects and cage number as random effects (42). Multiple tests were corrected for FDR.

IgA-Seq Analyses. Cecal content was collected from NOD and Eα16/NOD littermates, and frozen at −80 °C until further use. Sorting of IgA-bound cecal bacteria was carried out as previously described (27).

Preparation of Intestinal Cells for Immunologic Analysis and Flow Cytometry. Small intestine, cecum, and colon lamina propria cell suspensions and flow cytometry were prepared as previously described (39). The cecal patch was removed from the cecum before this tissue was used for the preparation of the lamina propria cell suspensions.

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