HHS Public Access

Author manuscript

Arthritis Rheumatol. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

Arthritis Rheumatol. 2016 September ; 68(9): 2151–2162. doi:10.1002/art.39681.

Perturbed Mucosal Immunity and Dysbiosis Accompany Clinical Disease in a Rat Model of Spondyloarthritis

Mark Asquith, PhD[*,1], **Patrick Stauffer, BS**[1], **Sean Davin, BS**[1], **Claire Mitchell, BS**[1], **Phoebe Lin, MD, PhD**[2], and **James T. Rosenbaum, MD**[1,2,3]

[1]Oregon Health and Science University, Division of Arthritis and Rheumatology,3181 SW Sam Jackson Park Rd, Portland, OR, USA, 97239

[2]Oregon Health and Science University, Casey Eye Institute, Portland, OR, USA, 97239

[3]Oregon Health & Sciences University, Ophthalmology and Medicine,3181 SW Sam Jackson Park Rd, Portland, OR, USA 97239

[4]Devers Eye Institute, 1040 Nw 22nd Ave, Suite 200, Portland, OR, USA, 97210

Abstract

Objective—The HLA-B27/β2 microglobulin (β2m) transgenic rat is a leading model of B27-associated spondyloarthopathy and disease is dependent on the presence of intestinal bacteria. We have shown previously that adult HLA-B27/β2m rats have an altered intestinal microbiota. In this study we sought to better define age-dependent changes to both mucosal immune function and dysbiosis in this model.

Methods—Intestinal contents were collected from wild type and HLA-B27/β2m+ rats post-weaning (3 and 6 weeks), at disease onset (10 wks) and after the establishment of disease (16 wks). Microbial community structure was determined by 16s sequencing and qRT-PCR. Mucosal and systemic Th1, Th17 and Treg responses were analyzed by flow cytometry, as was the frequency of IgA-coated intestinal bacteria. Intestinal expression of inflammatory cytokines and antimicrobial peptides (AMPs) was determined by qRT-PCR.

Results—An inflammatory cytokine signature and elevated AMP expression during the post-weaning period preceded the development of clinical bowel inflammation and dysbiosis in HLA-B27/β2m+ rats. An early and sustained expansion of the Th17 pool was specifically observed in cecal and colonic mucosa of HLA-B27/β2m rats. Strongly elevated *Akkermansia mucinphilia* colonization and IgA coating of intestinal bacteria was significantly associated with HLA-B27 expression and arthritis development.

Conclusions and Perspectives—HLA-B27/β2m expression in this rat model renders the host hyper-responsive to microbial antigens from infancy. Early activation of innate immunity and expansion of a mucosal Th17 signature are soon followed by dysbiosis in HLA-B27/β2m+ve animals. Perturbed mucosal immunity and dysbiosis strongly merit further study in both pre-diseased and diseased SpA patient populations.

[*]Corresponding author: Mark Asquith, PhD, Research Assistant Professor, Division of Arthritis and Rheumatic Diseases, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, Portland, OR 97239-3098, Phone: (503) 494-3371, Fax: (503) 494-6875, asquith@ohsu.edu.

INTRODUCTION

Spondyloarthropathies (SpAs) including ankylosing spondylitis (AS), reactive arthritis, and uveitis represent a spectrum of clinically related diseases with complex genetic, immunological and environmental etiology (1). MHC haplotype has the strongest genetic association with these diseases, with Human Leukocyte Antigen (HLA)-B27 greatly overrepresented in SpA patient populations (2). The strongest evidence that HLA-B27 mechanistically contributes to disease pathogenesis derives from the spondyloarthritis phenotype of transgenic rats that express multiple copies of the human B27-transgene in association with human β2-microglobulin (reviewed by (3)).

We have shown recently that HLA-B27/β2m rats have a distinct intestinal microbiota compared to strain-matched WT control animals (4). Pediatric and adult patients with SpA also exhibit an altered microbiota (termed dysbiosis) relative to healthy controls [5, 6]. This complements mounting evidence that the intestinal microbiota functionally impact SpA pathogenesis, as demonstrated by the absence of a disease phenotype in HLA-B27/β2m rats reared in germ free conditions or treated with antibiotics (7, 8). In humans, intestinal *Campylobacter, Salmonella, Shigella* and *Yersinia* spp. *or* urogenital *Chlamydia,* are established triggers of reactive arthritis, further indicating an intimate link between microbes and joint pathology (reviewed in (1). Moreover, up to 50% of SpA patients exhibit signs of microscopic bowel inflammation, and a significant proportion subsequently develop IBD (9, 10). AS patients also exhibit elevated levels of the fecal antimicrobial peptide (AMP) calprotectin – a biomarker of disease activity in Crohn's disease (CD) (11, 12).

From birth, an intricate relationship develops between the intestinal immune system and the resident gut microbiota (13). Exposure of intestinal microbes may elicit immune responses which promote homeostasis, for instance the induction of CD4+FoxP3+ Treg by Clostridial spp. (14). Alternatively, the induction of immune effectors may contribute to inflammation both locally and systemically such as the induction of Th17 signature effector cells by intestinal microbe segmented filamentous bacteria (SFB) that may contribute to the development of colitis, EAE and arthritis (14–16). The impact of HLA-B27 expression on the development of these responses therefore demands further insight.

In this study we sought to determine the age-dependent ontogeny of dysbiosis in HLA-B27/β2m rats and its relationship to immune status of the host. We examined the evolution of the mucosal immune response in HLA-B27/β2m animals from infancy (3 wks) until when animals develop bowel inflammation (10 wks) and arthritis (16+ wks). We observed that many parameters including AMP expression, microbiota-specific IgA response, Th17 and Treg responses are grossly disturbed in a HLA-dependent manner. Several (but not all) of these parameters were also disrupted prior to the onset of dysbiosis and HLA-B27 associated disease.

METHODS

Ethics Statement

All animal experiments were carried out under the ethical and experimental standards of the Association of Assessment and Accreditation of Laboratory Animal Care International and the Institutional Animal Care and Use Committee of Oregon Health & Sciences University.

Animals

The derivation of rats bearing the HLA-B27/hβ_2m (33-3 line) transgene has been described previously [17]. In brief, the 33-3 locus encodes 55 copies of B27 with 28 copies of the hβ_2m gene [18]. HLA-B27/hβ_2m transgenic rats on the Fischer 344 background (F33-3 line) were maintained at OHSU by crossing hemizygous females with founder WT F344 males (Jackson labs). Initial breeders for the F33-3 line were a generous gift of Maxime Breban. Both F344 33-3 offspring and WT littermate controls were separated post weaning and singly housed. All animals were maintained under specific pathogen free conditions and fed a diet of standard laboratory chow.

For longitudinal analysis of the microbiota, stool samples were collected weekly from F33-3 animals and littermate controls and stored at −80C. For longitudinal assessment of joint inflammation, a semi-quantitative scoring system was adopted. Each limb of the rat was assessed for redness and swelling. Each limb was scored 0.0, 0.5 or 1.0 and the sum of the four limbs is the noted score. A score of 0.0 is given for no disease, a score of 0.5 for minor swelling and/or redness of the joint, and a score of 1.0 for a red and swollen joint.

Sample collection

Animals aged 3–20 weeks were euthanized and tissues collected using sterile instruments. To collect mucosal samples, luminal contents were removed using phosphate buffered saline (PBS) and the remaining tissue vigorously swabbed to remove mucosally-associated microbes. Swabs and adjacent tissue were snap frozen and stored at −80C for subsequent analysis of microbial DNA or host RNA expression respectively.

Assessment of intestinal inflammation

Animals were sacrificed at the indicated ages and cecum and three colon sections (proximal/mid/distal) were fixed in 4% formalin. Sections were paraffin embedded, cut into 5μm sections and stained with hematoxylin and eosin. Sections were then scored in a blinded fashion using a scoring system described previously (19). This four parameter scoring system encompasses epithelial hyperplasia/goblet cell depletion, leukocytic infiltration of the lamina propria, markers of severe inflammation and total area of the intestinal section affected. Each parameter is scored 0–3, for a total score of 12. Scores for colon represent the mean scores of the three colon sections collected.

Analysis of microbial colonization by quantitative real-time PCR

Please see Supplementary Methods and Supp. Table 1 for full details. In brief, microbial DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) and phylum- (*Firmicutes, Proteobacteria*) or species- (*Akkermansia*

muciniphila) specific primers used as indicated to perform quantitative real-time PCR (qRT-PCR). The ΔΔCt relative quantification method was used as described previously (4), using the 16s rRNA gene as a reference gene.

Analysis of AMP and cytokine expression

Please see Supplementary Methods for full details. RNA was isolated from snap frozen tissue using Trizol Reagent (Life Technologies, Carlsbad, CA) and cDNA synthesized using High Capacity cDNA Reverse Transcription kit (Life Technologies). Taqman Gene Expression Assays (Life Technologies) were used for RT-qPCR determination of antimicrobial peptide mRNA expression. Published primer sequences were used to determine cytokine gene expression (for details see Supp. Table 2). Gene expression data was normalized to HPRT expression.

Lymphocyte isolation and flow cytometry

Preparations of spleen and MLN suspensions were performed as described previously (20). Intestinal lamina propria cells were isolated from either cecal or colonic tissue by EDTA incubation, collagenase/DNAaseI digestion and Percoll gradient (see (20) and Supplementary Methods). For analysis of cellular cytokine production, 10^6 cells were incubated per well of a 96 well plate at 37C for 4h in supplemented RPMI (3% FBS) containing phorbol myristate acetate/ionomycin and Brelfeldin A. Unstimulated controls were included with Brelfeldin A alone.

For flow cytometric analysis cells were incubated with cell surface antibodies (see below) and AQUA Live/Dead stain (Invitrogen, San Diego, CA) prior to fixation, permeabilization and intracellular staining with buffers from the FoxP3/Transcription Factor Staining Buffer Set (eBioscience, San Diego, CA). Cell surface antibodies used were CD3, CD4 and TCRβ and intracellular antibodies used were IL-17, IFNγ (cytokine panel) and FoxP3 and Helios (Treg panel). For antibody clones, conjugates and suppliers see Supp. Table 3. All flow cytometry samples were acquired with a BD Fortessa instrument (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (TreeStar, Ashland, OR). Frequencies of cytokine-producing cells represent the stimulated sample value minus that of its unstimulated control.

Bacterial IgA coating assay

To determine the frequency of IgA-coated bacteria, we modified an existing protocol (see (21)). Full details are provided in Supplementary Methods. In brief bacterial suspensions were prepared from fecal pellets and stained with mouse anti-Rat IgA (Acris antibodies, San Diego, CA), followed by Brilliant Violet 421 conjugated goat anti-mouse Ig (BD Bioscience). Cells were then co-stained with nuclear stain SYTO BC (Life Technologies) prior to PFA fixation and acquisition on a BD Fortessa instrument. Frequencies represent those of IgA+ cells amongst total SYTO BC +ve cells minus that of isotype controls.

Statistical Analysis

Statistical analysis to compare histological scores, bacterial colonization, gene expression and immune cell frequencies of WT vs transgenic animals was performed with PRISM software (GraphPad, San Diego, CA). Nonparametric data were compared using Mann-

Whitney U test and parametric data were compared using student's t test unless specified otherwise. Repeated measures ANOVA was used to compare longitudinal bacterial colonization. Linear regression was used for correlation analysis of *Akkermansia muciniphila* colonization and cecal mRNA expression of inflammatory cytokines.

RESULTS

Age-dependent development of dysbiosis in HLA-B27/β2m rats

To determine the impact of age on the intestinal microbiota in HLA-B27/β2m +ve rats, we analyzed the cecal microbiota of post-weaning (3 wks), juvenile (6 wks), adult (10 wks) and older animals (16+ ws) compared to littermate controls. WT and B27+ animals exhibited a similar microbiota post weaning (3–6 wks), yet exhibited significant changes to the intestinal microbiota by 10 wks of age, with a significant decrease in the relative abundance of Firmicutes spp., and a significant expansion of both Proteobacteria spp. and bacterium *Akkermansia muciniphila (Am)* (Fig. 1A–C). This HLA-B27 -dependent dysbiotic phenotype was also highly significant in older animals (16+ wks).

During the post-weaning period (3–6 wks), changes in the intestinal microbiota were particularly dynamic with age, albeit not significantly impacted by their HLA-B27/β2m expression. Strikingly, at 3 wks of age both HLA-B27 and WT controls exhibited a dysbiotic-like phenotype most closely resembling that of older HLA-B27/β2m animals. Since all animals were derived from transgenic HLA-B27/β2m mothers (see methods), these data are consistent with high exposure to maternal microbes during infancy. Nonetheless, by 6 wks, all animals exhibited a low relative abundance of *Am* and Proteobacteria spp. and a high relative abundance of Firmicutes spp. (Fig. 1A–C). This indicates that despite early exposure to and later development of a dysbiotic microbiota, HLA-B27/β2m animals may transiently exhibit a 'normal' intestinal microbiota at 6 wks of age.

In light of these findings, we examined the temporal relationship between a dysbiotic microbiota and the development of bowel inflammation (Fig. 1D). B27+ animals developed severe typhlitis and moderate colitis commencing at 10 weeks of age (Fig. 1D), with chronic intestinal inflammation persisting in older animals (16+ wks). By contrast, younger B27+ animals (3–6 wks) were free from overt clinical bowel inflammation in the first weeks post weaning (Fig. 1D).

HLA-B27 expression leads to early changes in inflammatory cytokine and AMP expression

We hypothesized that dysbiotic changes in HLA-B27/β2m+ animals might be preceded by local changes in immune mediators that may modulate the microbiota. Thus we examined mRNA levels of inflammatory cytokines and antimicrobial peptides in cecal tissue by RT-qPCR. We observed significantly elevated expression of Th17 signature cytokines, IL-23 and IL-17A (Fig. 2), in addition to Th1 signature cytokine, IFNγ, in 10 wk and 16 wk old HLA-B27/β2m+ animals concomitant with the establishment of clinical bowel inflammation (Fig. 1,2). These later time points were also accompanied by significantly elevated expression of mRNA for canonical innate cytokines IL-1β, TNFα and IL-8 (Fig. 2). Moderately, albeit significantly increased levels of IFNγ and IL-17 were also detected at 6

wks. Interestingly, at the earliest time point in our study (3 wks) we observed significantly increased expression of TNFα and IL-1β in HLA-B27/β2m+ animals relative to controls (Fig. 2). These latter findings indicate that HLA-B27/βm expression at an early age may trigger innate immune hyper-responsiveness in the gut, coinciding with the presence of a dysbiotic microbiota.

To further examine host factors impacting the microbiota that may be dysregulated in a HLA-B27-depedent manner, we also measured expression of anti-microbial peptides (AMPs). Strikingly, cecal mRNA expression of S100A8 and RegIIIγ was significantly increased in HLA-B27/β2m animals relative to age-matched controls (Fig. 3A,B). Interestingly these differences were observed prior to the development of clinical bowel inflammation at 6 wks, with S100A8 expression also significantly increased at weaning (3 wks). Expression of these mediators was elevated with increasing disease severity, but expression was also higher in non-diseased HLA-B27/β2m animals vs WT controls (Supp. Fig. 1). Expression of all AMPs measured however was not universally increased in HLA-B27/β2m+ animals. Cathelicidin-related Antimicrobial Peptide (cRAMP) exhibited equivalent expression in HLA-B27/β2m+ and WT control rats from 3–10 wks of age and was significantly decreased at 16 wks (Fig. 3C).

HLA-B27 expression drives an early expansion of mucosal Th17 cells and a perturbed Treg compartment

To further explore the evolving host immune response to the intestinal microbiota, we next examined the development of Th17 responses in HLA-B27/β2m+ animals from infancy to adulthood. At 10 wks, coincident with the development of clinical bowel inflammation (Fig. 1D), IL-17+CD4+ Th17 cells were greatly expanded in the intestinal mucosa (cecum and colon) of HLA-B27/β2m + animals, and also significantly expanded in MLN and spleen (Fig. 4A). At 16 wks, a significant expansion of mucosal and systemic Th17 cells was also observed. By contrast, a minimal Th17 response was observed at all tissue sites at weaning irrespective of genotype (3wks). Strikingly however, an early expansion of mucosal Th17 cells was observed in HLA-B27/β2m animals at 6wks, particularly in cecum. These findings indicate HLA-B27 expression drives an early expansion of mucosal Th17 cells, with site-specific differences to the degree of Th17 induction along the large intestinal tract.

We hypothesized that the observed perturbations in mucosal immunity might be accompanied by a dysregulated homeostatic response. To examine this, we again determined the frequency of CD4+ FoxP3+ Treg both mucosally and systemically (Fig. 4B). Notably, HLA-B27 rats exhibited a markedly altered distribution of FoxP3+ Treg compared to WT controls, with a reduced frequency of cecal CD4+FoxP3+ T cells at 16wks of age. By contrast, the frequency of CD4+FoxP3+ T cells in the draining MLN was highly increased at later time points in HLA-B27/β2m+ve animals. At 16wks, a modest yet significant increase in CD4+FoxP3+ T cell frequency was also observed in spleen. We did not detect HLA-B27 dependent differences in Treg accumulation in younger animals (3 and 6 wks, Fig. 4B), with the exception of a reduced cecal Treg frequency at the 3wk time point.

An elevated frequency of IgA-coated bacteria is associated with both HLA-B27 expression and arthritis

Since secretory IgA plays a critical role in shaping host immunity and the intestinal microbiota, we next employed a flow-cytometry based approach to enumerate the frequency of IgA-coated bacteria in HLA-B27/β2m animals relative to WT littermate controls (Fig. 5A–C). Notably, HLA-B27 expression was accompanied by a log-fold increase in the frequency of IgA-coated fecal bacteria in the oldest animals in our study (16 wks). This robust expansion of IgA-coated bacteria was a relatively late event in the evolving immune response to the microbiota, only beginning to emerge at 10 wks (Fig. 5C). Since arthritis onset typically occurs at 16 wks or older in Fischer 33-3 HLA-B27/β2m transgenic rats, and there was a low incidence of arthritis in our cross-sectional study of IgA-coating (2/9 animals in the 16wk age group, data not shown), we next probed archival stool samples from age-matched arthritic and non-arthritic HLA-B27/β2m rats using this IgA-coating assay (see Supp. Table 4 for ages and disease scores). Strikingly, there was a significantly increased frequency in fecal bacteria coated with IgA in arthritic vs non-arthritic animals (Fig. 5D). By contrast to the marked IgA coating observed in HLA-B27/β2m rats in these experiments, we found minimal coating of gut microbes with IgG in control experiments (Supp Fig. 2).

Akkermansia muciniphila colonization correlates with the severity of bowel inflammation and incidence of joint inflammation in HLA-B27/β2m animals

Recently, separate microbiome surveys in pediatric and adult SpA groups reported that *Akkermansia muciniphila* is over-represented, at least in a subset of patients (22). We therefore examined how *Am* colonization levels related to the spectrum of HLA-B27 dependent disease in the Fischer 33-3 transgenic rat. Cecal colonization levels by *Am* in HLA-B27/β2m animals showed a strong positive correlation with local mRNA expression levels of inflammatory cytokines IFNγ, IL-17A and IL-23 (Fig 6A–C), correlations that were not observed on the broader phylum level (Supp Fig. 3). To examine the relationship between *Am* colonization and the development of joint inflammation, we analyzed archived fecal samples that we had collected longitudinally from transgenic rats that either developed an arthritic or non-arthritic phenotype (Fig. 6D). Strikingly, HLA-B27/β2m animals that progressed to arthritis exhibited significantly higher *Am* colonization longitudinally relative to HLA-B27/β2m or WT animals that remained disease-free. Together these findings indicate that *Am* colonization is a potential microbial indicator of the severity of bowel inflammation and the development of joint inflammation in HLA-B27/β2m +ve rats.

DISCUSSION

Recently we have reported HLA-B27 expression profoundly shapes the intestinal microbiota in multiple strains of HLA-B27 transgenic rats, a leading model of spondyloarthropathy [4]. Subsequently, it has been reported that AS patients have a distinct ileal microbiota community profile compared to healthy controls and a dysbiotic phenotype is observed in the stool of pediatric SpA patients (5, 6, 22). To date, however, no study of SpA has examined temporal changes to the intestinal microbiota and their relationship to the evolving mucosal immune response.

In this study of Fischer 33-3 HLA-B27/β2m rats, we found HLA-B27 dependent changes in the intestinal microbiota were largely manifest with the onset of clinical bowel inflammation, with dysbiotic changes most evident at the later time points of our cross-sectional study (10wks and 16wks). Thus, HLA-B27 driven local inflammation may be a central driver of these dysbiotic changes. Previously, however, we have shown that adult Lewis 21–3×283–2 rats also develop a dysbiotic phenotype, despite the absence of overt bowel inflammation in this HLA-B27 transgenic line (4). This indicates active bowel inflammation is not a pre-requisite for disruption to the normal gut microbiota. Nonetheless, we observed that multiple immune parameters were significantly altered even in pre-diseased HLA-B27/β2m animals, including elevated transcripts for AMP and inflammatory cytokines and a large expansion of the local CD4+ Th17 compartment. These are plausible mechanisms through which HLA-B27 expression may alter the intestinal microbiota in those HLA-B27+ve AS patients or transgenic rat lines that exhibit histologically normal gut architecture.

An established paradigm is that gene-environment interactions underpin the pathogenesis of AS, CD and related diseases. Indeed the clear requirement for the microbiota to drive disease in HLA-B27 transgenic animals (7) underscores this environmental component in disease pathogenesis. We note that WT animals remained healthy throughout our study, despite harboring a gut microbial community in infancy similar to their dysbiotic HLA-B27/β2m+ mothers. Interestingly, another study by Rehaume et al found that in the murine SKG model of SpA, transfer of microbiota from ZAP70-deficient mice transferred disease to healthy WT recipients (23). By contrast, neonatal exposure of WT pups to maternal HLA-B27/β2m+ feces during weaning did not induce disease in recipient animals in our study. This indicates that in the SKG model, it is the microbiome driving disease, rather than the ZAP70 variant. HLA-B27/β2m expression however appears to be strictly required for genetic priming of the host immune system to induce disease. This reaffirms the idea that a dysbiotic microbiota alone may be insufficient to induce disease and HLA-B27/β2m expression is also a key requirement for the loss of gut homeostasis and pathogenesis. Indeed, identification of mechanisms underlying the ‘recovery’ of a dysbiotic to normal microbiota in WT animals may also help identify some of these homeostatic pathways. These pathways are incompletely understood, but could involve bactericidal mechanisms (e.g. phagocytosis or AMP production) or provision of metabolites such as fucose to promote outgrowth of benign commensal species (reviewed in (1)).

The mutualistic relationship between the microbiota and immune system develops from the first exposure of the naïve intestinal tract to microbial antigens at birth. Our findings that TNFα, IL-1β and AMP expression are all elevated in infant (3wk old) cecal tissue of HLA-B27/β2m animals relative to age-matched controls indicate HLA-B27 expression renders the gut immunologically hyper-responsive from an early age. This exaggerated innate immune response is followed by marked latter expansion in mucosal expression of Th1 and Th17 signature cytokines (IFNγ and IL-17/IL-23) – whose upregulated colonic mRNA expression in HLA-B27/β2m rats has also been described previously (24). Several studies indicate the intestinal lamina propria may be a key inductive site for Th17 cells (25, 26). Consistently, ~30% of lamina propria T cells were IL-17+ve in HLA-B27/β2m animals at only 6 wks of age, whereas expansion of extra-intestinal Th17 cells (MLN and spleen) was more moderate

and observed at later time points. These latter findings mirror those of Glatigny et al, in which a significant expansion in the frequency of popliteal and mesenteric lymph node Th17 cells commenced at 3 months of age in HLA-B27/β2m relative to controls (27). Recently it has also been shown that arthritogenic T cells with high migratory capacity are primed in the gut of K/BxN mice (16, 28). Thus, local induction of Th17-like cells in HLA-B27/β2m rats may also potentiate effector populations capable of exiting the gut and inducing inflammation at distal sites.

Our study also revealed that HLA-B27 expression may also perturb the relative tissue distribution of CD4+FoxP3+ T cells. We found the inflamed intestine of older HLA-B27/β2m animals exhibited a loss of this homeostatic cell population (Figure 4B). This finding was somewhat surprising given reports of their increased frequency in the gut of AS and CD patients [29]. Either site-specific (e.g. ileum vs large intestine) or species-specific differences may explain this discrepancy. By contrast to the gut however, HLA-B27 expression was associated with a marked accumulation of CD4+FoxP3+ T cells in the MLN of inflamed animals. Indeed, a previous study reported that CD4+CD25+ T cells accumulated in the MLN of HLA-B27+ rats relative to WT controls (30). Previously however, it has been reported that HLA-B27 transgenic rats exhibit a loss of tolerogenic CD103+ $CD172^{lo}$ DCs that traffic to the MLN – a site of Treg induction (31, 32). The expanded Treg population in the MLN was therefore unexpected. A plausible hypothesis to examine is that further to dysregulated DC trafficking, HLA-B27 expression may also disrupt homing of Treg from the MLN to the intestinal lamina propria.

Loss of the dominant Firmicutes phylum is an observation reported in both IBD patients and rodent colitis models (33-35). Interestingly, *Clostridial* spp. of this phylum are known inducers of intestinal Treg (14), potentially through high production of short-chain fatty acids [36]. These metabolites may promote epithelial fitness and limit inflammatory cytokine production by enterocytes and leukocytes (Reviewed by (37)). The loss of Firmicutes in a HLA-B27 dependent manner may thus promote breakdown of host-microbiota homeostatic mechanisms and inflammation. Conversely, *Proteobacteria* outgrowth was also observed in HLA-B27 rats in this and a previous study (38), and is dysbiotic phenotype reported in IBD (39). This phylum includes *E.coli* and other *Enterobacterial spp.* which may intimately associate with the intestinal mucosa and induce local inflammation (reviewed in (40)). Nonetheless, given that these dysbiotic changes were concomitant with bowel inflammation in HLA-B27 rats, we cannot determine whether they are a cause or a result of HLA-B27 dependent disease.

We also examined expression mRNA levels of soluble mediators known to shape the intestinal microbiota. We found both innate (AMPs RegIIIγ and S100A8) and adaptive mediators (secretory IgA) were strongly impacted by HLA-B27 expression. Interestingly, elevated fecal calprotectin (S100A8) levels have been reported in the stool of both AS patients and first degree relatives compared to controls (11, 12, 41), mirroring their increased cecal mRNA expression found here. We propose that early and sustained elevated expression of AMPs such as S100A8 and RegIIIγ may deplete benign intestinal microbes, creating a niche exploited by pathogenic organisms. Contrasting these early changes in AMP expression, we found the dramatic HLA-B27 dependent increase in the frequency of IgA-

coated bacteria was a relatively late event. This could merely reflect differences in sensitivity of RT-qPCR and flow cytometric assays used to measure these mediators or be the result of chronic intestinal inflammation in older animals. Alternatively, this may herald the onset of HLA-B27 dependent pathology beyond the gut. Indeed, a higher frequency of IgA-coated bacteria was observed in arthritic vs non-arthritic HLA-B27+ rats, despite the presence of penetrant bowel inflammation in all older animals.

Elevated IgA responses to the enteric flora are a feature of IBD and AS patients. For instance, bacterial flagellin is a dominant antigen in CD, and elicits robust IgA responses (42). Moreover, elevated IgA responses have been reported against yeast *Saccharomyces cerevisiae* (ASCA), a diagnostic marker in CD, in SpA patients (43, 44). Whereas these studies used ELISA to assay IgA titers, we adopted a flow-based assay to identify IgA-coated microbes. In WT animals, we found relatively few microbes coated with high levels of IgA. This is consistent with reports that under homeostatic conditions, commensals elicit an IgA response with relatively low affinity and specificity (reviewed by (45, 46). By contrast, particularly robust IgA responses are elicited against pathogenic gut microbes and recently it has been reported that IgA coating identifies colitogenic bacteria in CD (21). This provides a strong impetus to establish whether IgA coating may be a marker of colitogenic or arthritogenic bacteria in the HLA-B27 rat, or whether there is a direct mechanistic link with disease pathology.

In the final part of our study, we observed a strong correlation between *Akkermansia muciniphila* colonization and B27-dependent inflammation of gut and joint. Interestingly increased *Am* colonization is a shared dysbiotic feature of HLA-B27/β2m rats and SpA patient subpopulations (5, 22). Increased carriage of *Am* has also been reported in psoriatic arthritis and in IBD pouchitis (47, 48). *Am* is as a mucin-degrading bacterium that closely associates with enterocytes (49). Interestingly, HLA-B27/β2m+ rats produce increased intestinal mucin (50), a substrate that may favor *Am* colonization. Moreover, *Am* is an immunologically active microbiota constituent, inducing expression of a panoply of immune genes including those involved in AMP synthesis and antigen presentation (51, 52). Therefore enhanced *Am* colonization may not be merely be secondary to changes to the gut milieu but contribute to the inflammatory cascade in SpA-associated disease. We interpret this model however with caution. For instance, vancomycin is reported to induce a striking outgrowth of *Am* (53), despite its ability to attenuate bowel inflammation in HLA-B27/β2m rats (8). Moreover, *Am* has previously been shown to be protective in the DSS IBD model that DSS treatment reduces its carriage (54). Thus, if *Am* is driving HLA-B27-associated disease it may be doing so in concert with other members of the gut microbiota.

The authors note that a limitation of the current study was that we were unable to include an MHC-class I control (for instance the HLA-B7 transgenic rat). The use of such strains in future studies would help clarify which of the microbiota and immunological changes described here were specific to HLA-B27 or potentially associated with expression of other human HLA haplotypes. Further analogous studies in human SpA populations and controls would also prove highly useful in this regard.

In summary we report the progressive dysbiosis of HLA-B27/β2m+ rats is marked by dynamic changes to the intestinal immune environment. These include dysregulated expression of AMPs and increased production of bacterial-specific IgA. These changes are accompanied by enhanced mucosal innate and adaptive cytokine expression, with local perturbation of effector and regulatory T cell populations (Th17 and Tregs). Intriguingly, both *Am* colonization and IgA-coating of gut microbes were significantly associated with arthritis development in HLA-B27/β2m rats. Thus, further dissection of dybiotic changes and perturbed mucosal immune function may prove enlightening to further understand SpA pathogenesis. Moveover, strategies to target the intestinal microbiota therapeutically, for instance dietary intervention or fecal microbiota transplant (FMT) may limit these HLA-B27 dependent immune changes and offer novel therapeutic opportunities for the clinical management of HLA-B27 associated spondyloarthropathies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by grants from the Collins Medical Trust and the Medical Research Foundation of Oregon to M.A. PL is supported by a grant from the NIH (K08EY0229480). JTR is supported by the Stan and Madelle Rosenfeld Family Trust and the William and Mary Bauman Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare no commercial interests for this study.

References

1. Asquith M, Elewaut D, Lin P, Rosenbaum JT. The role of the gut and microbes in the pathogenesis of spondyloarthritis. Best practice & research Clinical rheumatology. 2014; 28(5):687–702. [PubMed: 25488778]
2. Lopez de Castro JA. HLA-B27 and the pathogenesis of spondyloarthropathies. Immunol Lett. 2007; 108(1):27–33. [PubMed: 17129613]
3. Taurog JD, Maika SD, Satumtira N, Dorris ML, McLean IL, Yanagisawa H, Sayad A, Stagg AJ, Fox GM, Le O'Brien A, et al. Inflammatory disease in HLA-B27 transgenic rats. Immunol Rev. 1999; 169:209–223. [PubMed: 10450519]
4. Lin P, Bach M, Asquith M, Lee AY, Akileswaran L, Stauffer P, Davin S, Pan Y, Cambronne ED, Dorris M, et al. HLA-B27 and Human beta2-Microglobulin Affect the Gut Microbiota of Transgenic Rats. PLoS One. 2014; 9(8):e105684. [PubMed: 25140823]
5. Costello ME, Ciccia F, Willner D, Warrington N, Robinson PC, Gardiner B, Marshall M, Kenna TJ, Triolo G, Brown MA. Intestinal dysbiosis in ankylosing spondylitis. Arthritis & rheumatology. 2014
6. Stoll ML. Gut microbes, immunity, and spondyloarthritis. Clin Immunol. 2015
7. Taurog JD, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernandez-Sueiro JL, Balish E, Hammer RE. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. J Exp Med. 1994; 180(6):2359–2364. [PubMed: 7964509]
8. Rath HC, Schultz M, Freitag R, Dieleman LA, Li F, Linde HJ, Scholmerich J, Sartor RB. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. Infect Immun. 2001; 69(4):2277–2285. [PubMed: 11254584]
9. Baeten D, De Keyser F, Mielants H, Veys EM. Ankylosing spondylitis and bowel disease. Best practice & research Clinical rheumatology. 2002; 16(4):537–549. [PubMed: 12406426]
10. Van Praet L, Van den Bosch FE, Jacques P, Carron P, Jans L, Colman R, Glorieus E, Peeters H, Mielants H, De Vos M, et al. Microscopic gut inflammation in axial spondyloarthritis: a multiparametric predictive model. Ann Rheum Dis. 2013; 72(3):414–417. [PubMed: 23139267]

11. Klingberg E, Carlsten H, Hilme E, Hedberg M, Forsblad-d'Elia H. Calprotectin in ankylosing spondylitis--frequently elevated in feces, but normal in serum. Scand J Gastroenterol. 2012; 47(4): 435–444. [PubMed: 22229862]
12. Matzkies FG, Targan SR, Berel D, Landers CJ, Reveille JD, McGovern DP, Weisman MH. Markers of intestinal inflammation in patients with ankylosing spondylitis: a pilot study. Arthritis research & therapy. 2012; 14(6):R261. [PubMed: 23194008]
13. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014; 157(1): 121–141. [PubMed: 24679531]
14. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011; 331(6015):337–341. [PubMed: 21205640]
15. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2011; 108(Suppl 1):4615–4622. [PubMed: 20660719]
16. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, Littman DR, Benoist C, Mathis D. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010; 32(6):815–827. [PubMed: 20620945]
17. Taurog JD, Maika SD, Simmons WA, Breban M, Hammer RE. Susceptibility to inflammatory disease in HLA-B27 transgenic rat lines correlates with the level of B27 expression. J Immunol. 1993; 150(9):4168–4178. [PubMed: 8473755]
18. Fert I, Cagnard N, Glatigny S, Letourneur F, Jacques S, Smith JA, Colbert RA, Taurog JD, Chiocchia G, Araujo LM, et al. Reverse interferon signature is characteristic of antigen-presenting cells in human and rat spondyloarthritis. Arthritis & rheumatology. 2014; 66(4):841–851. [PubMed: 24757137]
19. Izcue A, Hue S, Buonocore S, Arancibia-Carcamo CV, Ahern PP, Iwakura Y, Maloy KJ, Powrie F. Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis. Immunity. 2008; 28(4):559–570. [PubMed: 18400195]
20. Uhlig HH, McKenzie BS, Hue S, Thompson C, Joyce-Shaikh B, Stepankova R, Robinson N, Buonocore S, Tlaskalova-Hogenova H, Cua DJ, et al. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. Immunity. 2006; 25(2):309–318. [PubMed: 16919486]
21. Palm NW, de Zoete MR, Cullen TW, Barry NA, Stefanowski J, Hao L, Degnan PH, Hu J, Peter I, Zhang W, et al. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. Cell. 2014; 158(5):1000–1010. [PubMed: 25171403]
22. Stoll ML, Kumar R, Morrow CD, Lefkowitz EJ, Cui X, Genin A, Cron RQ, Elson CO. Altered microbiota associated with abnormal humoral immune responses to commensal organisms in enthesitis-related arthritis. Arthritis research & therapy. 2014; 16(6):486. [PubMed: 25434931]
23. Rehaume LM, Mondot S, Aguirre de Carcer D, Velasco J, Benham H, Hasnain SZ, Bowman J, Ruutu M, Hansbro PM, McGuckin MA, et al. ZAP-70 Genotype Disrupts the Relationship Between Microbiota and Host, Leading to Spondyloarthritis and Ileitis in SKG Mice. Arthritis & rheumatology. 2014; 66(10):2780–2792. [PubMed: 25048686]
24. DeLay ML, Turner MJ, Klenk EI, Smith JA, Sowders DP, Colbert RA. HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. Arthritis Rheum. 2009; 60(9):2633–2643. [PubMed: 19714651]
25. Goto Y, Panea C, Nakato G, Cebula A, Lee C, Diez MG, Laufer TM, Ignatowicz L, Ivanov II. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal th17 cell differentiation. Immunity. 2014; 40(4):594–607. [PubMed: 24684957]
26. Geem D, Medina-Contreras O, McBride M, Newberry RD, Koni PA, Denning TL. Specific microbiota-induced intestinal Th17 differentiation requires MHC class II but not GALT and mesenteric lymph nodes. J Immunol. 2014; 193(1):431–438. [PubMed: 24899505]
27. Glatigny S, Fert I, Blaton MA, Lories RJ, Araujo LM, Chiocchia G, Breban M. Proinflammatory Th17 cells are expanded and induced by dendritic cells in spondylarthritis-prone HLA-B27-transgenic rats. Arthritis Rheum. 2012; 64(1):110–120. [PubMed: 21905004]

28. Morton AM, Sefik E, Upadhyay R, Weissleder R, Benoist C, Mathis D. Endoscopic photoconversion reveals unexpectedly broad leukocyte trafficking to and from the gut. Proc Natl Acad Sci U S A. 2014; 111(18):6696–6701. [PubMed: 24753589]
29. Ciccia F, Accardo-Palumbo A, Giardina A, Di Maggio P, Principato A, Bombardieri M, Rizzo A, Alessandro R, Ferrante A, Principe S, et al. Expansion of intestinal CD4+CD25(high) Treg cells in patients with ankylosing spondylitis: a putative role for interleukin-10 in preventing intestinal Th17 response. Arthritis Rheum. 2010; 62(12):3625–3634. [PubMed: 20722024]
30. Araujo LM, Fert I, Jouhault Q, Labroquere K, Andrieu M, Chiocchia G, Breban M. Increased production of interleukin-17 over interleukin-10 by treg cells implicates inducible costimulator molecule in experimental spondyloarthritis. Arthritis & rheumatology. 2014; 66(9):2412–2422. [PubMed: 24909668]
31. Utriainen L, Firmin D, Wright P, Cerovic V, Breban M, McInnes I, Milling S. Expression of HLA-B27 causes loss of migratory dendritic cells in a rat model of spondylarthritis. Arthritis Rheum. 2012; 64(10):3199–3209. [PubMed: 22674414]
32. Cording S, Wahl B, Kulkarni D, Chopra H, Pezoldt J, Buettner M, Dummer A, Hadis U, Heimesaat M, Bereswill S, et al. The intestinal micro-environment imprints stromal cells to promote efficient Treg induction in gut-draining lymph nodes. Mucosal Immunol. 2014; 7(2):359–368. [PubMed: 23945546]
33. Sartor RB. Microbial influences in inflammatory bowel diseases. Gastroenterology. 2008; 134(2): 577–594. [PubMed: 18242222]
34. Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev. 2005; 206:260–276. [PubMed: 16048554]
35. Manichanh C, Borruel N, Casellas F, Guarner F. The gut microbiota in IBD. Nature reviews Gastroenterology & hepatology. 2012; 9(10):599–608. [PubMed: 22907164]
36. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013; 341(6145):569–573. [PubMed: 23828891]
37. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients. 2011; 3(10):858–876. [PubMed: 22254083]
38. Onderdonk AB, Richardson JA, Hammer RE, Taurog JD. Correlation of cecal microflora of HLA-B27 transgenic rats with inflammatory bowel disease. Infect Immun. 1998; 66(12):6022–6023. [PubMed: 9826389]
39. Gevers D, Kugathasan S, Denson LA, Vazquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song SJ, Yassour M, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe. 2014; 15(3):382–392. [PubMed: 24629344]
40. Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. IBD-what role do Proteobacteria play? Nature reviews Gastroenterology & hepatology. 2012; 9(4):219–230. [PubMed: 22349170]
41. Bjarnason I, Helgason KO, Geirsson AJ, Sigthorsson G, Reynisdottir I, Gudbjartsson D, Einarsdottir AS, Sherwood R, Kristjansson K, Kjartansson O, et al. Subclinical intestinal inflammation and sacroiliac changes in relatives of patients with ankylosing spondylitis. Gastroenterology. 2003; 125(6):1598–1605. [PubMed: 14724811]
42. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM. Bacterial flagellin is a dominant antigen in Crohn disease. J Clin Invest. 2004; 113(9):1296–1306. [PubMed: 15124021]
43. Hoffman IE, Demetter P, Peeters M, De Vos M, Mielants H, Veys EM, De Keyser F. Anti-saccharomyces cerevisiae IgA antibodies are raised in ankylosing spondylitis and wundifferentiated spondyloarthropathy. Ann Rheum Dis. 2003; 62(5):455–459. [PubMed: 12695160]
44. Torok HP, Glas J, Gruber R, Brumberger V, Strasser C, Kellner H, Marker-Hermann E, Folwaczny C. Inflammatory bowel disease-specific autoantibodies in HLA-B27-associated spondyloarthropathies: increased prevalence of ASCA and pANCA. Digestion. 2004; 70(1):49–54. [PubMed: 15308872]

45. Pabst O. New concepts in the generation and functions of IgA. Nat Rev Immunol. 2012; 12(12): 821–832. [PubMed: 23103985]

46. Slack E, Balmer ML, Fritz JH, Hapfelmeier S. Functional flexibility of intestinal IgA - broadening the fine line. Frontiers in immunology. 2012; 3:100. [PubMed: 22563329]

47. Morgan XC, Kabakchiev B, Waldron L, Tyler AD, Tickle TL, Milgrom R, Stempak JM, Gevers D, Xavier RJ, Silverberg MS, et al. Associations between host gene expression, the mucosal microbiome, and clinical outcome in the pelvic pouch of patients with inflammatory bowel disease. Genome Biol. 2015; 16:67. [PubMed: 25887922]

48. Scher JU, Ubeda C, Artacho A, Attur M, Isaac S, Reddy SM, Marmon S, Neimann A, Brusca S, Patel T, et al. Decreased bacterial diversity characterizes the altered gut microbiota in patients with psoriatic arthritis, resembling dysbiosis in inflammatory bowel disease. Arthritis & rheumatology. 2015; 67(1):128–139. [PubMed: 25319745]

49. Derrien M, Vaughan EE, Plugge CM, de Vos WM. Akkermansia muciniphila gen. nov., sp. nov. a human intestinal mucin-degrading bacterium. International journal of systematic and evolutionary microbiology. 2004; 54(Pt 5):1469–1476. [PubMed: 15388697]

50. Faure M, Moennoz D, Mettraux C, Montigon F, Schiffrin EJ, Obled C, Breuille D, Boza J. The chronic colitis developed by HLA-B27 transgenic rats is associated with altered in vivo mucin synthesis. Dig Dis Sci. 2004; 49(2):339–346. [PubMed: 15104381]

51. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A. 2013; 110(22):9066–9071. [PubMed: 23671105]

52. Derrien M, Van Baarlen P, Hooiveld G, Norin E, Muller M, de Vos WM. Modulation of Mucosal Immune Response, Tolerance, and Proliferation in Mice Colonized by the Mucin-Degrader Akkermansia muciniphila. Frontiers in microbiology. 2011; 2:166. [PubMed: 21904534]

53. Hansen CH, Krych L, Nielsen DS, Vogensen FK, Hansen LH, Sorensen SJ, Buschard K, Hansen AK. Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. Diabetologia. 2012; 55(8):2285–2294. [PubMed: 22572803]

54. Kang CS, Ban M, Choi EJ, Moon HG, Jeon JS, Kim DK, Park SK, Jeon SG, Roh TY, Myung SJ, et al. Extracellular vesicles derived from gut microbiota, especially Akkermansia muciniphila, protect the progression of dextran sulfate sodium-induced colitis. PLoS One. 2013; 8(10):e76520. [PubMed: 24204633]

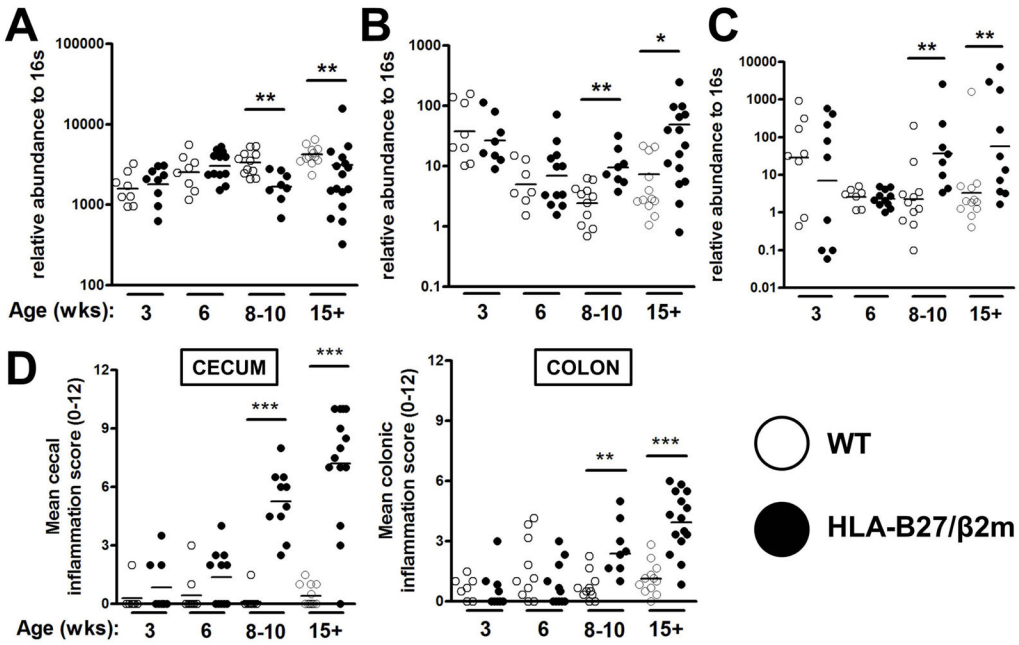

Figure 1. Development of intestinal inflammation and an altered intestinal microbiota in HLA-B27/β2m rats

Fischer 344 (33-3) HLA/B27/β2m transgenic and WT littermate control mice were sacrificed at the indicated time points and cecal mucosal colonization determined by RT-qPCR using phylum- or species-specific primers (A–C). Abundance of *Firmicutes* (A), *Proteobacteria* (B) and *Akkermansia muciphila* (C) were calculated relative to 16s (arbitrary units). (D) Cecal and colonic inflammation was assessed histologically. Each symbol represents a single animal, and the data were pooled from two to three independent experiments per time point (n=7–14 per age/genotype). Horizontal lines represent group means. *p < 0.05, ** p < 0.01, *** p < 0.001.

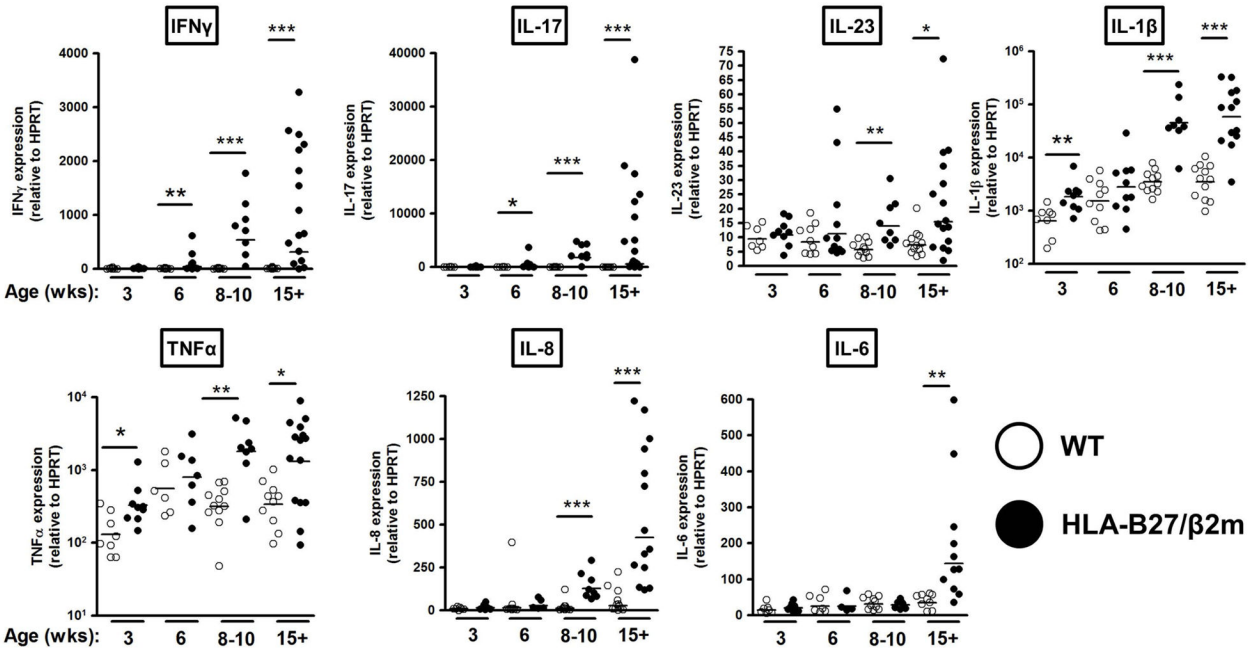

Figure 2. Cecal cytokine expression in WT and HLA-B27/β2m rats

Fischer 344 (33-3) HLA/B27/β2m transgenic and WT littermate control mice were sacrificed at the indicated time points and cecal cytokine mRNA expression determined by RT-qPCR. Expression level of (A) IFNγ, IL-17A, IL-23, IL-1β, TNFα,IL-8 and IL-6 was calculated relative to housekeeping gene HPRT. Each symbol represents a single animal, and the data were pooled from two to three independent experiments per time point (n=7–14 per age/genotype). Horizontal lines represent group means. *p < 0.05, ** p < 0.01, *** p < 0.001.

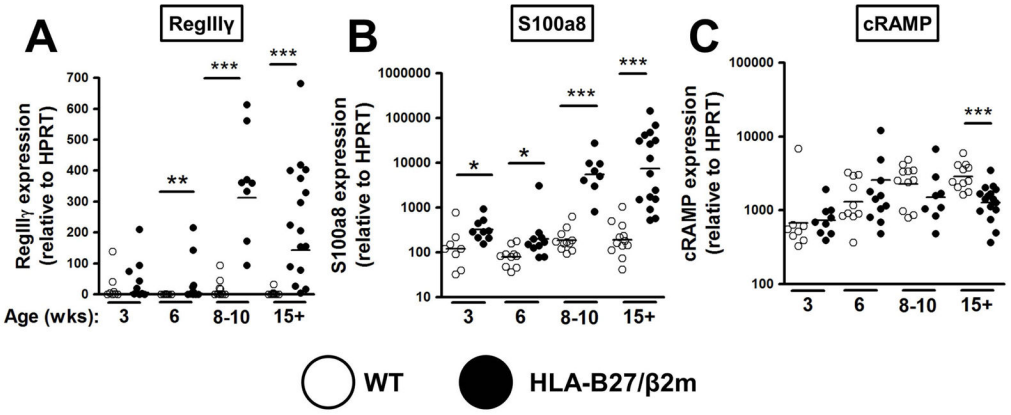

Figure 3. Cecal antimicrobial peptide (AMP) expression in WT and HLA-B27/β2m rats

Fischer 344 (33-3) HLA/B27/β2m transgenic and WT littermate control mice were sacrificed at the indicated time points and cecal AMP mRNA expression determined by RT-qPCR. Expression level of (A) RegIIIγ, (B) S100A8 (C) cRAMP was calculated relative to housekeeping gene HPRT. Each symbol represents a single animal, and the data were pooled from two to three independent experiments per time point (n=7–14 per age/genotype). Horizontal lines represent group means. *p < 0.05, ** p < 0.01, *** p < 0.001.

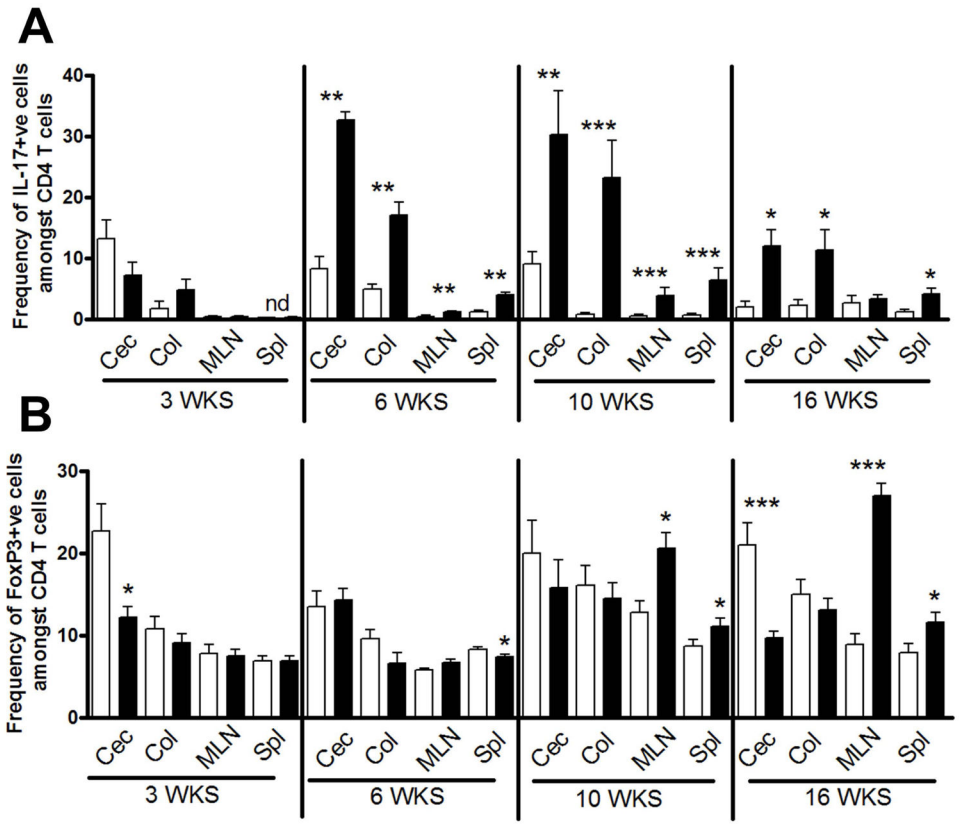

Figure 4. Disruption of the Th17/Treg compartment in HLA-B27/β2m rats

Fischer 344 (33-3) HLA/B27/β2m transgenic and WT littermate control mice were sacrificed at the indicated time points. Lymphocytes were isolated from cecal and colonic lamina propria, mesenteric lymph node (MLN) and Spleen and the frequency of (A) CD4+IL-17+ T cells and (B) CD4+FoxP3+ T cells was determined by flow cytometry (n=5-12 animals/genotype/time point). Data represents two to three pooled experiments per time point. Bars represent group means +/− SEM. *p < 0.05, ** p < 0.01, *** p < 0.001.

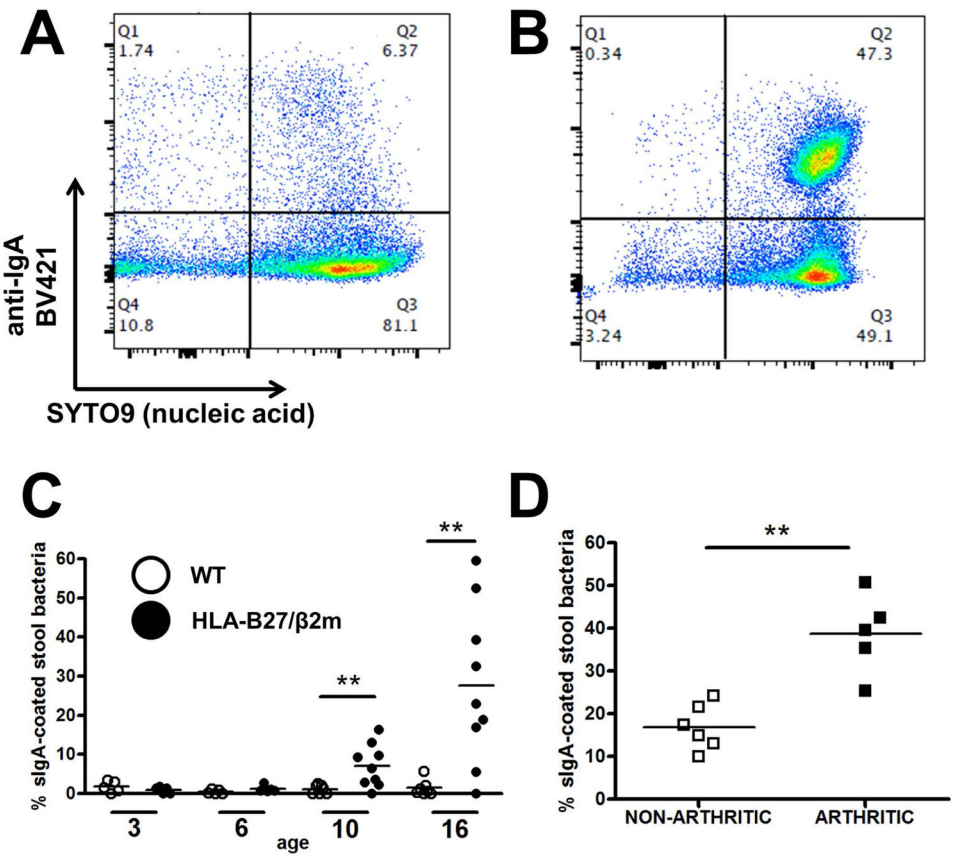

Figure 5. HLA-B27/β2m rats harbor an increased frequency of IgA-coated microbes

Feces were collected from Fischer 344 (33-3) HLA/B27/β2m transgenic and WT littermate control mice at the indicated time points. To enumerate the frequency of IgA coated bacteria, fecal contents were stained with SYTO BC nuclear stain and with anti-rat IgA. (A–B) Representative flow cytometric plots of IgA staining in A) WT and B) HLA/B27/β2m transgenic rats (16weeks). (C) The percentage of IgA+ve fecal bacteria at the indicated time points. (D) Feces from adult (16–20wk) arthritic and non-arthritic H;A-B27/β2M rats were analyzed for IgA coating (as in C). Each symbol represents a single animal, with data pooled from two independent experiments. ** $p < 0.01$.

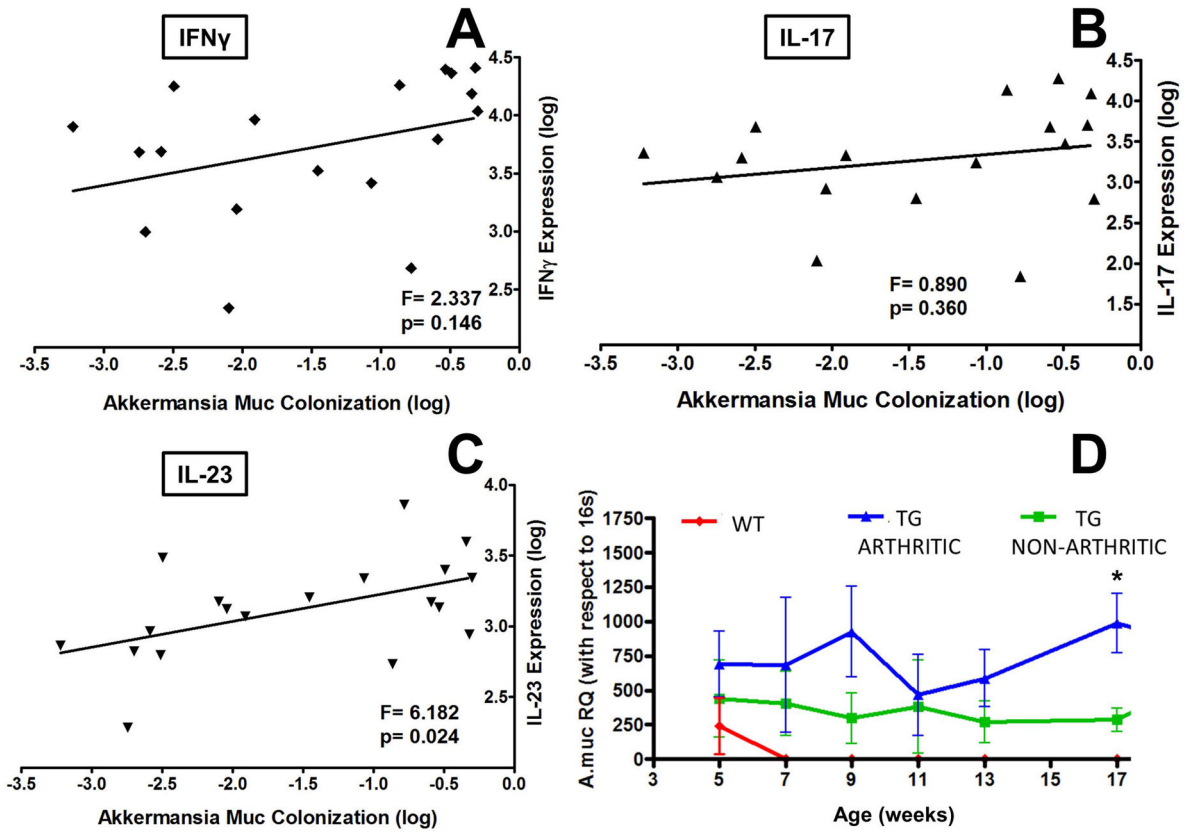

Figure 6. *Am* colonization correlates with the severity of bowel inflammation and development of arthritis in HLA-B27/β2m rats

(A–C) Linear regression was performed on log-transformed cecal mucosal *Akkermansia muciniphila* colonization (relative to 16s) and cecal mRNA expression of either (A) IFNγ, (B) IL-17 or (C) IL-23 (calculated relative to HPRT). F and p values shown on figure. Each symbol represents a single animal. (D) Longitudinal *Am* colonization (relative to 16s) was determined at the indicated time points in WT (red line) animals or HLA-B27/β2m animals that either progressed to arthritis (blue line) or remained non-arthritic (green line). Bars represent group means +/− SEM, n = 5–6 per group. *p < 0.05.