Dual IgA/IgG family autoantibodies from individuals at-risk for rheumatoid arthritis identify an arthritogenic strain of *Subdoligranulum*

Meagan Chriswell[1], Adam R. Lefferts[1], Michael Clay[2], Alex Hsu[3], Jennifer Seifert[1], Marie L. Feser[1], Cliff Rims[4], Michelle Bloom[3], Elizabeth A. Bemis[1], M. Kristen Demoruelle[1], Kevin D. Deane[1], Eddie A. James[4], Jane H. Buckner[4], William H. Robinson[3], V. Michael Holers[1], Kristine A. Kuhn[1]

[1]Division of Rheumatology, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, [2]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO. [3]Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, Palo Alto, CA. [4]Benaroya Research Institute, Seattle, WA

Hypothesis/Background

A growing body of evidence supports the mucosal origins hypothesis of Rheumatoid arthritis (RA), indicating that the nidus for RA pathogenesis may begin at mucosal tissues. Herein we utilize human plasmablast-associated antibodies to identify and isolate an arthritogenic strain of *Subdoligranulum* and demonstrate high level mechanisms by which it immunomodulates its host. We hypothesize that our ***Subdoligranulum* isolate 7 is targeted by antibodies polyreactive against synovial targets, and is capable of inducing intestinal immune responses that become systemic responses cumulating in antibody-dependent joint inflammation.**

Methods

Plasmablast-associated monoclonal antibodies (mAbs) were utilized as probes to determine binding against a diverse pool of commensal organisms

CD4+ T cells from individuals with RA were matched against *Subdoligranulum* strains 1 and 7, and CD154 and CD69 upregulation were determined

Germ free DBA/1 mice were monocolonized with *Subdoligranulum* isolates 1, 7, and *Prevotella copri*. The mice were monitored for arthritis development weekly, and for microscopic joint damage after day 35.

Joint slides from monocolonized mice were stained for endogenous complement C3, IgG, and IgA deposition, and were scored in a blinded fashion.

Colons from monocolonized mice were fixed and sectioned. ILF counts were performed.

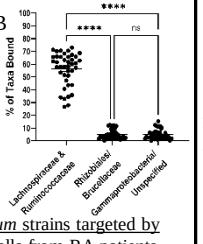
Total serum IgA was determined in monocolonized mice 14 days after bacterial gavage. Titers of autoantibodies against a variety of RA-relevant autoantigens was determined.

Serum from monocolonized mice was transferred intraperitoneally into healthy mice, and then mice were monitored weekly for arthritis development.

Figure 1: IgA/IgG family plasmablast-derived mAbs cross-react with RA-relevant antigens and bind families *Lachnospiraceae* and *Ruminococcaceae*.

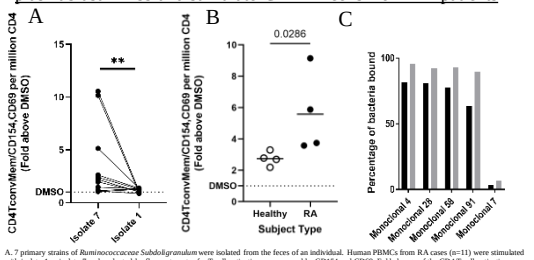
A

	Serum CCP+ at-risk Individuals				Early RA Individuals		Total (%)
Subject ID	1	2	3	4	5	6	
Number of mAbs	18	18	18	24	7	9	94
Percent reactive against RA-relevant antigens	100	100	100	100	100	100	100
Percent bacterially reactive	66.7	83.3	55.6	70.8	42.9	11.1	61.7



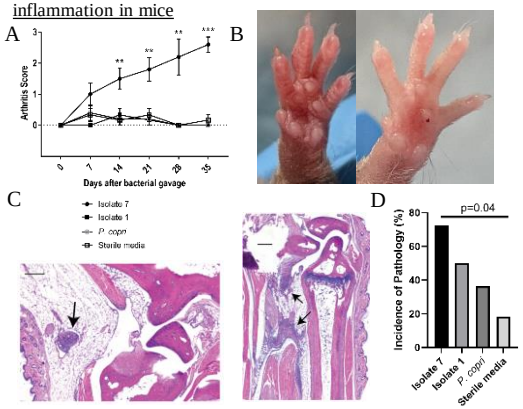
A, 94 plasmablast-derived mAbs from at-risk (n=4) and eRA (n=2) individuals were screened for cross-reactivity against a broadly representative pool of fecal bacteria created from feces from individuals with eRA (n=5), at-risk (n=8) and healthy controls (n=5). The fecal pool was exposed to each antibody and analyzed by flow cytometry. B. The three most bound taxa from each PB-mAb are represented out of the total bacteria bound (x-axis). The percent of total bacteria bound is quantified for each mAb (y-axis) and shown as round symbols for the individual mAbs and mean ± SEM as bars. P<0.0001 by one-way ANOVA with Tukey's post-test.

Figure 2: *Ruminococcaceae Subdoligranulum* strains targeted by plasmablast mAbs and stimulate CD4+ T cells from RA patients



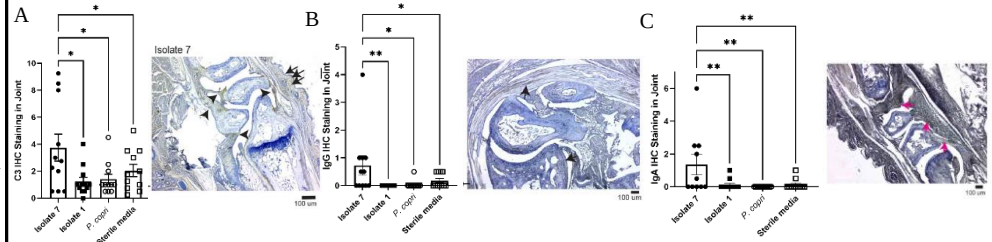
A. 7 primary strains of *Ruminococcaceae Subdoligranulum* were isolated from the feces of an individual. Human PBMCs from RA cases (n=11) were stimulated with isolate 1 or isolate 7 and evaluated by flow cytometry for T cell activation as measured by CD154 and CD69. Fold change of the CD4 T cell activation response relative to DMSO control (y-axis) is displayed for isolates 1 and 7 (x-axis). **,P<0.01, non-parametric Wilcoxon matched-pairs signed rank test. B. Isolate 7 specific responses among T cells was tested, comparing cells derived from RA cases to healthy control T cells. Fold change of the CD4 T cell activation as determined by CD154 and CD69 relative to DMSO (y-axis) is displayed, comparing healthy control cells to RA case cells (x-axis). P=0.0286, Mann-Whitney test. C. Isolates 1 and 7 were matched against four selected PB-mAbs (numbers 4, 28, 58, and 91) that bound highly to other various patterns of *Ruminococcaceae* and *Lachnospiraceae* species to verify that they targeted the strains. They were also matched against a control mAb (number 7) that was previously found to not bind bacteria.

Figure 3: *Subdoligranulum* strain stimulates joint swelling and inflammation in mice



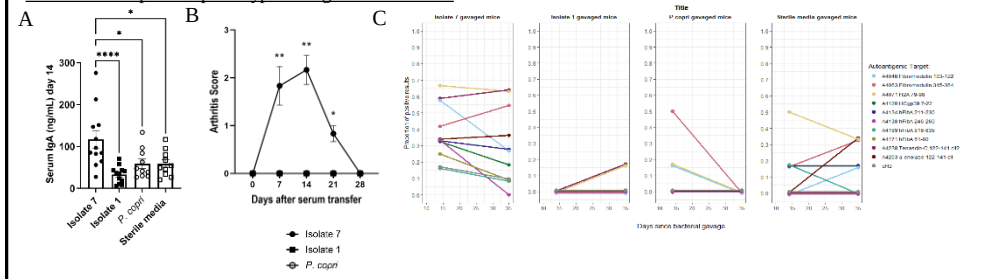
A. *Subdoligranulum* isolates 1 and 7 as well as *Prevotella copri* or sterile media were gavaged separately into germ-free DBA/1 mice (n=6 isolate 1, n=6 isolate 7, n=5 *P. copri*, and n=6 sterile media). Mice were observed weekly for 35 days for the development of joint swelling and assessed a score based on the number of joints affected. The mean ± SEM score is shown (y-axis) over time after bacterial gavage (x-axis). **,P<0.01and ***,P<0.001, unpaired t-test. B. Representative photographs of paws from the treatment groups are shown to demonstrate the swelling observed in mice monoc-colonized with isolate 7. C. Paw histology was assessed by a pathologist in a blinded fashion. D. Displayed is the incidence of pathology (y-axis) separated by treatment group (x-axis). P=0.04, Fisher's exact test.

Figure 4: *Subdoligranulum* monocolonized mice exhibit increased IgG, IgA, and complement C3 deposition in joints



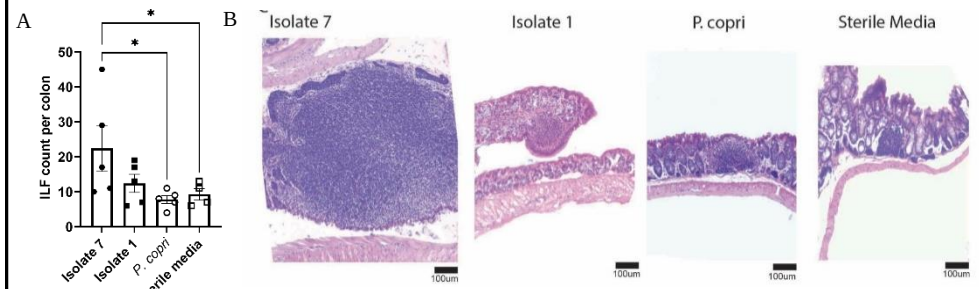
A. IHC for the C3 component of complement was performed on decalcified paw sections. C3 staining intensity scores are displayed (y-axis) separated by treatment group (x-axis). Symbols represent individual mice while bars are the mean ± SEM. *,P<0.05, one-way ANOVA with Bonferroni post-test. A representative section for isolate 7 is displayed, with deposition indicated by arrows; the scale bar represents 100μm. B. IgG deposition in decalcified paw sections was assessed by IHC. Intensity scores with symbols as individual mice and bars as mean ± SEM are displayed (y-axis), separated by treatment group (x-axis). *,P<0.05 and **P<0.01, one-way ANOVA with Tukey post-test. A representative section is displayed, with deposition indicated by arrows; the scale bar represents 100μm. C. IgA deposition in decalcified paw sections was assessed by IHC and intensity scores are displayed (y-axis), separated by treatment group (x-axis). Symbols represent individual mice while bars are the mean ± SEM. **P<=0.01, one-way ANOVA with Tukey post-test. A representative section is displayed, with deposition indicated by arrows; the scale bar represents 100μm. (n=12 isolate 1 gavaged, n=11 isolate 7 gavaged, n=11 *P. copri* gavaged, and n=11 sterile media gavaged, across two experiments).

Figure 5: *Subdoligranulum* isolate 7 causes development of increased serum IgA and systemic RA-related autoantibodies that are able to recapitulate phenotype through serum transfer



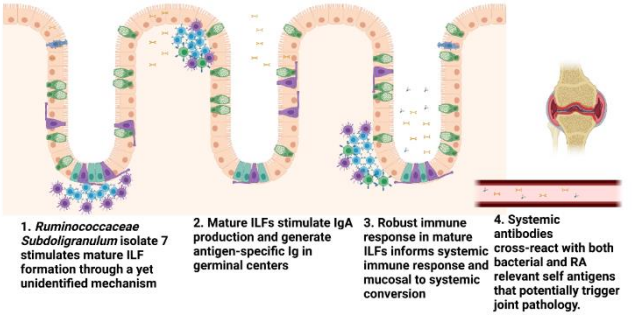
A. Serum from mice mono-colonized with either isolate 1 (n=6), isolate 7 (n=12), or *P. copri* (n=6), or given sterile media (n=6) was collected at days 14 and 35 after gavage.The total serum IgA at 14 days after gavage was determined by ELISA; serum IgA is displayed (y-axis) against treatment group (x-axis). Symbols represent individual mice while bars are the mean ± SEM. *,P<0.05 and ****,P<0.0001, Kruskal-Wallis test with Dunn's post-test. B. Pooled day 35 serum from mice mono-colonized with isolate 1, isolate 7, and *P. copri* was injected into healthy germ-free DBA/1 mice, and these mice were monitored for the development of joint swelling. The mean clinical score ± SEM (n=6 isolate 7 serum transfer, n=6 isolate 1 serum transfer, n=7 *P. copri* serum transfer) is shown relative to time after-serum transfer (x-axis). P<0.05, Kruskal-Wallis with Dunn's post-test. C. Sera was analyzed on a planar array containing ~350 citrullinated and native peptides for autoantigens relevant in RA. A cutoff for positivity was established at the 80th percentile of autoantibody reactivity for individual samples on this assay (1.5 relative units) and the proportion of murine samples meeting or exceeding this threshold (11 antigens as displayed by) at each timepoint is shown (y-axis). Each treatment group is shown from left to right.

Figure 6: *Subdoligranulum* isolate 7 causes development of intestinal isolated lymphoid follicles at colonic epithelium



A. Colon histology from each group was analyzed. The number of isolated lymphoid follicles (ILFs) per colon (y-axis) separated by treatment group (x-axis) is shown. Symbols represent individual mice while bars are the mean ± SEM. *,P<0.05, one-way ANOVA with Tukey post-test. B. Representative colon histology containing ILFs in each group are shown. Scale bars represent 100μm.

Graphical Abstract



Conclusions

Ruminococcaceae Subdoligranulum isolate 7 is targeted by human T cells and plasmablast-associated antibodies

Subdoligranulum isolate 7 is able to stimulate arthritis development characterized by C3, IgG, and IgA deposition

There is significant mature ILF development in the colons of isolate 7 gavaged mice

Serum autoantibodies develop in *Subdoligranulum* isolate 7 gavaged mice, and are capable of transferring the phenotype through serum transfer.

Disclosures and Acknowledgements

The authors have no relevant financial conflicts of interest.
Funding for this work includes RRF Rheumatology Future Physician Scientist Award and NIH/NIAID U01 AI101981