Rheumatoid Arthritis

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Abbreviations

ACPA	Anti-cyclic citrullinated peptide antibody
CRA	Chronic rheumatoid arthritis
DAS	Disease activity score
DC	Dendritic cells
HC	Healthy control
IL	Interleukin
JIA	Juvenile idiopathic arthritis
MMP	Matrix metalloproteinase
NORA	New-onset rheumatoid arthritis
NSAID	Nonsteroidal anti-inflammatory drug
OA	Osteoarthritis
PAD	Peptidylarginine deiminase
PD	Periodontal disease
PPAD	*P. gingivalis* peptidylarginine deiminase
RA	Rheumatoid arthritis
SE	Shared epitope
SRP	Scaling and root planning
TNFi	Tumor necrosis factor inhibitor

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Introduction

The concept that rheumatoid arthritis (RA) could be mediated by infections is more than 100 years old, since Bailey suggested that the disease was likely mediated by bacterial toxins and indicated that the offending bacterium may reside in the gastrointestinal tract [1]. Indeed, the RA infection theory was the rationale for the development of sulfasalazine in the 1940s [2] as well as for several of the early trials evaluating antibiotics as a therapeutic tool (Table 15.1). Over the ensuing decades, the concept that RA was mediated by infections largely fell out of favor, because no single organism was clearly identified using candidate organism approaches. The pendulum has swung back. Beginning with the study by Vaahtovuo et al. [3], multiple investigators have used culture-independent technology to query mucosal populations at several different body surfaces, finding abnormalities that in many cases have been remarkably consistent and which lead to the conclusion that the oral and enteric microbiota predispose to the development of RA and the formation of its hallmark antibody, anti-cyclic citrullinated peptide antibodies (ACPAs).

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Table 15.1 Antibiotic trials in RA. [12–14]

Study	Patient population	Blinding	Dose (*n*)[a]	Comparator (*n*)	Duration	Outcome
Antimycobacterials—rifampicin						
Borg et al. [83]	Duration < 12 months, naïve to DMARDs or CS	Unclear	600 mg QD [9]	HCQ 400 mg QD [7]	12 months	Favored HCQ group in multiple markers of disease activity
Fluoroquinolones—levofloxacin						
Ogrendik [84]	On MTX	DB	500 mg daily [38]	PCB [38]	6 months	Favored levofloxacin group in multiple markers of disease activity
Macrolides—clarithromycin						
Ogrendik [85]	Duration < 3 years, failed 1–4 DMARDs	DB	500 mg QD [41]	PCB [40]	6 months	ACR20 24/41 (59%) CM vs 13/40 (33%) PCB, $p < 0.001$
Saviola et al. [86]	Not taking DMARDs	SB	500 mg BID × 14 days, then QD + MTX, prednisone [16]	Placebo + MTX, prednisone [16]	1 month	ACR70 10/16 (62%) CM vs 4/16 (25%) PCB, $p = 0.033$
Macrolides—roxithromycin						
Ogrendik [87]	Duration <1 year, naïve to DMARDs	DB	300 mg QD [16]	PCB [15]	3 months	ACR20 response in 12/16 (75%) roxithromycin vs 3/15 (20%) PCB, $p = 0.002$
Ogrendik and Karagoz [88]	Failed 1–4 DMARDs	DB	300 mg daily [50]	PCB [50]	6 months	ACR20 response in 30/50 (60%) roxithromycin 17/50 (34%) PCB, $p = 0.009$
Tetracyclines—doxycycline						
Sreekanth et al. [89]	Not taking DMARDs	SB	100 mg BID [15]	MTX 7.5 mg weekly [14]	6 months	Non-statistically significant improvement in MTX arm in several markers of active arthritis
St. Clair et al. [90]	Duration 6 months–12 years	DB	200 mg IV daily × 3 weeks, then weekly [10]	PCB [10]	4 weeks	No differences between the groups
Van der Laan et al. [91]	Stable DMARD therapy for ≥10 months	DB, crossover	50 mg BID [48][b]	PCB [18]	36 weeks	No change in a number of markers of active arthritis
Pillemer et al. [92]	Any	DB	300 mg IV daily [10]	PCB IV [13]	12 weeks	ACR50 in 1/10 (10%) doxycycline vs 0/13 with PCB ($p = 0.43$)
O'Dell et al. [75]	Duration <1 year, naïve to DMARDs	DB	100 mg BID [24] or 20 mg BID [18]	Placebo [24]	2 years	ACR50 response in 10/24 (42%) high-dose, 7/18 (39%) low-dose, and 3/24 (12%) PCB, $p = 0.03$
Tetracyclines—minocycline						
Kloppenburg et al. [93]	Failed ≥1 DMARD	DB	100 mg BID [40]	PCB [40]	26 weeks	Favored minocycline in multiple markers of disease activity

Tilley et al. [94]	Failed ≤1 DMARD for inefficacy	DB	50 mg BID [109]	PCB [110]	48 weeks	Improvements in joint swelling (54% vs 39%, $p = 0.023$) and tenderness (56% vs 41%, $p = 0.021$)
O'Dell et al. [95]	Duration <1 year, naïve to DMARDs	DB	100 mg BID [23]	PCB [23]	6 months	Response seen in 15/23 (65%) minocycline vs 3/23 (13%) PCB, $p < 0.001$
O'Dell et al. [96]	Duration <1 year, naïve to DMARDs	DB	100 mg BID [30]	HCQ 200 mg BID [30]	2 years	ACR50 response in 18/30 (60%) minocycline vs 10/30 (33%) HCQ, $p = 0.04$
Tetracyclines—tetracycline						
Skinner et al. [97]	Any	DB	250 mg QD [15]	PCB [15]	54 weeks	No differences between the groups
Tetracyclines plus lincosamides—tetracycline/clindamycin						
Gompels et al. [98]	Duration >6 months, on DMARD	SB	Clindamycin 900 mg IV weekly × 1 month, then q2 weeks + tetracycline 250 mg 3× weekly [11]	No additional therapy [10]	12 months	ACR20 of 5/11 (45%) antibiotics vs 0/10 controls, $p = 0.04$
Smith et al. [99]	Failed ≥1 DMARD	DB	Clindamycin variable dose + tetracycline BID 3× weekly [12]	PCB [8]	25 weeks	ACR20 in 2/12 (17%) antibiotics vs 0/8 PCB, NS
Nitroimidazole—metronidazole						
Harkness et al. [100]	Any	DB	400 mg BID [10]	PCB [10]	6 weeks	No differences in markers of active arthritis
Marshall et al. [101]	Any	DB	400 mg TID [24]	PCB [26]	24 weeks	Improved articular index and pain score in metronidazole completers, but >75% withdrew due to AEs
Nitroimidazole—ornidazole						
Ogrendik et al. [102]	Not specified	Double	1000 mg daily [53] or 500 mg daily [55]	PCB [52]	3 months	Favored both groups of ornidazole in multiple markers of disease activity
Sulfonamides—sulfamethoxazole alone						
Ash et al. [103]	Not taking DMARDs	SB, crossover	2 g daily [23]	PCB [23]	6 months	Favored SFX group in multiple markers of disease activity

(continued)

Table 15.1 (continued)

Study	Patient population	Blinding	Dose (*n*)[a]	Comparator (*n*)	Duration	Outcome
Sulfonamides—sulfamethoxazole/trimethoprim						
Wojtulewski et al. [104]	Not taking DMARDs	DB	40 mg/kg BID [24]	Ketoprofen 50 mg TID [23]	8 weeks	No differences between the groups
Sulfonamides—sulfasalazine						
Neumann et al. [105]	No DMARDs in 3 months prior to enrolment	Unclear	2000 mg daily [31]	D-penicillamine 500 mg daily [32]	4 months	SSZ was equivalent to D-penicillamine
Pullar et al. [106]	Naïve to DMARDs	DB	3000 mg daily [30]	PCB [30]	24 weeks	Favored SSZ group in multiple markers of disease activity
Pinals et al. [107]	No DMARDs in 3 months prior to enrolment	DB	1500 mg BID [50]	PCB [36]	15 weeks	Favored SSZ group in multiple markers of disease activity
Williams et al. [108]	Duration >6 months, no DMARDs	DB	500 mg QID [69]	PCB [51]	37 weeks	ITT analysis favored SSZ group over placebo in multiple markers of disease activity
Hannonen et al. [109]	Duration <1 year, naïve to DMARDs	DB	2000 mg daily [38]	PCB [40]	48 weeks	Favored SSZ group in multiple markers of disease activity

[a]All drugs were administered orally unless indicated otherwise

[b]Due to crossover design, not all of the 48 were on the medication for the entire treatment period

BID twice daily, *CM* clarithromycin, *DB* double-blinded, *DMARD* disease-modifying antirheumatic drug, *ITT* intention to treat, *MTX* methotrexate, *NS* not significant, *PCB* placebo, *QD* daily, *QID* four times daily, *SB* single-blinded (assessor only), *SFX* sulfamethoxazole, *SSZ* sulfasalazine, *TID* three times daily

Fecal Microbiota in RA

Multiple studies have evaluated the contents of the fecal microbiota in RA (Table 15.2). There is substantial heterogeneity in the published studies, primarily in the methodology used to identify the bacteria, the geographic location of the subjects, the use of immunomodulatory medications in the RA patients, and the source of the controls. The first three studies to evaluate the microbiota as a whole used fecal culture followed by various analytic techniques to identify anaerobic and aerobic organisms, as well as to identify a limited number of specific organisms through traditional methods [4–6]. These studies were limited in their ability to identify the vast majority of the bacteria present in the intestinal tract, and not surprisingly, few differences emerged. Shinebaum [4] reported increased *C. perfringens* in RA patients, a finding that was subsequently thought to be secondary to the use of nonsteroidal anti-inflammatory drugs (NSAIDs) on the basis of the observation that RA patients and osteoarthritis (OA) patients on NSAIDs had similar burden of this organism and both patient populations had a higher abundance of *C. perfringens* as compared to OA patients not taking NSAIDs [6]. Severijnen reported higher frequency of what was termed "coccoid rods" in RA patients [5]. None of these studies identified any bacteria that were lower in patients.

Although still widely used in clinical medicine, culture is a suboptimal modality to differentiate all of the components of a complex community of bacteria. It is generally cited that only about 20% of intestinal bacteria can be cultured [7]. Although the number may in fact be higher [8], culture and identification is nevertheless a highly labor-intensive approach; it has been estimated that to culture and identify the fecal community of bacteria would take about one person-year of laboratory effort [9]. In contrast, the process of sequencing of 16S ribosomal DNA and its analysis can be completed in a few days.

Thus, as the technology became available, genetic tools were used to compare the fecal microbiota of RA patients and controls. The first such study to do so was published by Vaahtovuo et al. [3]. This study was nevertheless limited by the use of specific genetic probes, rather than pan-bacterial markers that have since become state of the art. In addition, this early study was possibly limited by the use of patients with fibromyalgia as controls, as it has not been established whether their microbiota is representative of healthy adults. They observed four probe sets of bacteria to be reduced in RA ([1] *Bacteroides*/*Porphyromonas*/*Prevotella*, [2] *B. fragilis*, [3] *Bifidobacterium*, [4] *Eubacterium rectale–Clostridium coccoides* group) and did not identify any elevated probes. Today, we recognize that inclusion of both *Bacteroides* and *Prevotella* in a single probe set is a limitation, as these two genera constitute two distinct enterotypes, which tend to be inversely correlated with one another [10].

All subsequent studies used either sequencing of the 16S ribosomal DNA, whole-genome sequencing, or a combination of these approaches. As discussed elsewhere (Chap. 3), these approaches constitute far more comprehensive and relatively unbiased approaches to query the microbiota. The first of these metagenomics studies was the groundbreaking work published by Scher and colleagues in 2013 evaluating populations of subjects with new-onset RA (NORA), long-standing or chronic RA (CRA), and healthy controls (HC) [11]. This study also included subjects with psoriatic arthritis, which is the topic of a different chapter (Chap. 18). One of the key findings was a striking increase in the abundance of a single organism, *Prevotella copri*, which had a fecal abundance upwards of 50% in some subjects and greater than 5% in 33/44 (75%) of NORA subjects compared to 6/28 (21%) of HC. Fecal carriage of *P. copri* was higher in RA patients without versus with the shared epitope (SE), a 5 amino acid sequence motif in residues 70–74 of the HLA-DRβ chain (QKRAA, QRRAA, or RRRAA) that is the genetic factor that confers the highest risk for RA susceptibility [12]. This latter finding suggests that the abundance of *P. copri* above a certain threshold may be needed to overcome the lack of genetic predisposition to RA. Interestingly, the abundance of *P. copri* in subjects with CRA was similar to that of healthy controls.

Table 15.2 Fecal microbiota in RA

Study	Methods	Population	Depleted bacteria	Enriched bacteria	Additional findings	Location
Shinebaum et al. [4]	Culture and gram-staining	NORA ($n = 25$), HC ($n = 25$)	None	*Clostridium perfringens*	Associated with disease activity	Edinburgh, UK
Severijnen et al. [5]	Culture and gram-staining	CRA ($n = 10$), HC ($n = 10$)	None	Coccoid rods, aerobic organisms	Differences in aerobes were abrogated when pts on SSZ and d-PCN were removed	Rotterdam, Netherlands
Dearlove et al. [6]	Culture and gram-staining	NORA ($n = 22$), OA ($n = 48$)	None	None	Association with *C. perfringens* likely due to NSAIDs	Harrogate and Leeds, UK
Vaahtovuo et al. [3]	Targeted 16S	NORA ($n = 51$), FM ($n = 40$)	Four probes: [1] *Bacteroides/Porphyromonas/Prevotella,* [2] *B. fragilis,* [3] *Bifidobacterium*, [4] *Eubacterium rectale–Clostridium coccoides* group	None	None	Turku, Finland
Scher et al. [11]	16S and WGS	NORA ($n = 44$), CRA ($n = 26$), HC ($n = 28$)	*Bacteroides*	*Prevotella copri*	NORA patients had lower alpha diversity, depending on measure used	New York City, NY, USA
Zhang et al. [15]	WGS	NORA ($n = 94$), HC ($n = 80$), relatives ($n = 17$)	*K. pneumoniae, Megamonas hypermegale, Sutterella wadsworthensis, Bifidobacterium bifidum*	*Bacteroides*, *Eggerthella lenta*, *Clostridium asparagiforme*		Beijing, China
Chen et al. [14]	16S	CRA ($n = 40$), FDR ($n = 15$), HC ($n = 17$)	*Faecalibacterium*	Actinobacteria phylum; *Collinsella* and *Eggerthella*	Lower alpha diversity in CRA patients	Rochester, MN, USA
Maeda et al. [13]	16S	NORA ($n = 17$), HC ($n = 14$)	*None*	*Prevotella copri*		Osaka, Japan

CRA chronic rheumatoid arthritis, *FDR* first-degree relatives, *FM* fibromyalgia, *HC* healthy controls, *NORA* new-onset rheumatoid arthritis, *OA* osteoarthritis, *WGS* whole-genome sequencing

A subsequent study of NORA patients likewise suggested a role for intestinal *P. copri* in the etiopathogenesis of RA. Maeda et al. studied 17 subjects with NORA and 14 HC [13]. Principal component analysis of the sequencing of the 16S rDNA identified four clusters. One dominated by *Prevotella* was comprised only of RA patients. Most of the *Prevotella* sequences aligned closely with *P. copri*, and patients in the *Prevotella* cluster had elevated inflammatory markers when compared to patients in the remaining clusters.

A Chinese study of NORA patients did not identify significant differences in the abundance of fecal *P. copri* between patients and controls, indicating that geographic differences in genetics and diet likely also play important roles in determining microbial contributions to arthritis. In this study, 94 NORA patients and 80 HC underwent metagenomic shotgun sequencing. Taxa abundant in RA patients included *Eggerthella lenta* and *Clostridium asparagiforme*, while those abundant in controls included *Klebsiella pneumoniae*, *Megamonas hypermegale*, *Sutterella wadsworthensis*, and *Bifidobacterium bifidum*. Longitudinal evaluation of treated RA participants showed that baseline levels of some bacteria, particularly those containing certain virulence factors, were predictive of response to therapy. Using repeat specimens from 40 patients following initiation of therapy, the authors showed that changes in the gut microbiota did not correlate very well with response to therapy.

A North American study of CRA patients showed RA patients to be deficient in fecal *Faecalibacterium prausnitzii* and abundant for rare bacteria within the Actinobacteria phylum, primarily *Collinsella* and *Eggerthella* [14]. The latter finding is consistent with the study by Zhang et al. [15]. As discussed elsewhere (Chap. 19) in this textbook, *F. prausnitzii* has been shown to be decreased in adult and pediatric patients with inflammatory bowel disease [16], as well as in children with enthesitis-related arthritis [17, 18]. Its role in arthritis has been attributed to a variety of potential factors, such as its effects of development of regulatory T cells [19] and on the health of the enterocytes [20].

In summary, multiple studies have evaluated the fecal microbiota in RA patients. All of the studies that used sequencing methods to identify bacteria have identified substantial differences between RA patients and controls. Moreover, two of them, despite geographic heterogeneity, demonstrated depletion of *Bacteroides* [3, 11] and two showed increased abundance of *P. copri* [11, 13], findings which have not been observed in patients with CRA [11, 14]. The only exception to these general findings was a study conducted in China, in which *Bacteroides* was enriched in RA patients [15]. Additional commonalities described in this body of work include that two of these studies demonstrated expansion of a rare genus called *Eggerthella* [14, 15]. Finally, both studies that reported on the within-group (alpha) diversity of the samples demonstrated decreased diversity in RA patients, although in one of these studies, this finding was dependent upon the metric used [11, 14].

Several studies provided mechanisms by which the associated bacteria may predispose to arthritis. For example, one of the findings by Chen et al. was that a rare genus within the Actinobacteria phylum, *Collinsella*, was enriched in RA patients [14]. As part of the study, the authors introduced this organism into the collagen-induced arthritis model, finding that addition of *Collinsella* increased the frequency albeit not the severity of arthritis. They also found that mouse dendritic cells (DC) pre-cultured with *Collinsella* demonstrated more robust responses to collagen as compared to DC not cultured with *Collinsella* and that *Collinsella* increased the permeability of the CACO-2 intestinal cell line. Taken together, they proposed that a combination of decreased *Faecalibacterium* and increased *Collinsella* resulted in increased intestinal permeability, potentially permitting microbial components to enter the lamina propria and trigger dysfunctional immunity. Likewise, Scher et al. demonstrated that colonization of antibiotic-depleted mice with *P. copri*, the most abundant organism in their study, resulted in increased colitis induced by dextran sulfate [11]. Maeda et al. used fecal transplant to test the ability of *Prevotella* to induce arthritis in SKG mice

injected with zymosan, finding that microbiota containing *Prevotella* were associated with the development of arthritis while microbiota lacking *Prevotella*—whether derived from RA patients or healthy controls—did not [13]. The *Prevotella*-exposed mice also had increased numbers of CD4+ and CD4 + IL-17+ T cells in the large intestine, and T cells derived from regional lymph nodes in these mice showed enhanced Th17 responses compared to T cells derived from mice exposed to control microbiota.

Thus, studies of the fecal microbiota in RA patients indicate expansion of *P. copri* in NORA patients and also show in animal systems that *P. copri* is pro-inflammatory and immunogenic. Pianta et al. demonstrated that *P. copri* is immunogenic in humans as well [21]. They used liquid chromatography mass spectroscopy to identify the peptidome from HLA-DR+ antigen-presenting cells. Among them was a peptide that matched to a portion of a 27-kD protein from *P. copri* (Pc-p27). Production of interferon-gamma following in vitro exposure to this peptide was observed in T cells from 17/40 (42%) of RA patients compared to 0/15 healthy controls and 0/10 patients with Lyme arthritis. Likewise, RA patients demonstrated increased levels of IgA antibodies against both the peptide and whole bacteria, with the levels of these antibodies correlating with those of inflammatory cytokines. Thus, *P. copri* is not only abundant in NORA but also appears to trigger mucosal immune reactions.

While there is compelling evidence that *P. copri* is likely involved in the initiation of RA, there are still multiple unanswered questions. It is not known what drives the expansion of *Prevotella*, nor what factors cause it evidently to return to normal in patients with long-standing disease. Would prevention of this expansion of *P. copri* be able to prevent this disease from starting, and would eradication of *P. copri* be a therapeutic option? The latter seems unlikely, in light of the absence of any studies showing expansion of this organism in patients with long-standing disease. Additionally, if *P. copri* induces mucosal immunity and inflammation, as the study by Scher suggested [11], why is subclinical gut inflammation a rare finding in patients with RA, as compared to patients with spondyloarthritis [22, 23]?

Periodontal Disease and Associated Microbiota in RA

The gut is not the only habitat that has been associated with RA; the oral microbiota may also play an important role in the disease, particularly in the context of periodontal disease (PD). PD is fundamentally an infectious and inflammatory process [24, 25]. An early step in the initiation of PD is the development of a biofilm consisting of oral bacteria. This biofilm permits the expansion of pathogenic organisms, such as *Porphyromonas gingivalis*, that are not ordinarily present on the gingival surface in significant quantities [25]. *P. gingivalis* is a gram-negative anaerobic coccobacillus that can both elude host immune responses and cause local tissue destruction [25]. Deep sequencing of the gingival microbiota revealed that *P. gingivalis* is only present in subjects with PD, even among RA patients [26]. The host responds to the microbial challenge by generating an immunologic response, consisting of various innate and adaptive mediators of inflammation. This results in plaque formation and local gingival inflammation. As this progresses, the connective tissue attachment to the tooth is damaged, followed by the development of bone destruction [24]. Treatment of periodontitis typically consists of a procedure called scaling and root planning (SRP), which consists of physical removal of the plaque, which is the nidus of the inflammatory process [27].

There is abundant epidemiologic evidence of an association between RA and PD [26, 28–31]. For example, a cross-sectional study conducted through the National Health and Nutrition Examination Survey III consisting of 4461 North American participants showed that RA patients were more likely to have PD compared to those without RA (OR 1.82 following adjustment for multiple potential confounders, including smoking status, 95% CI 1.04–3.20) [28]. Likewise, a

cross-sectional study of 852 non-smoking adults in India referred for periodontal evaluation showed an incidence of RA of 4.4%, compared to 1% in the general population [29]. Small studies have found such an association as well [26, 30, 31], including those that were limited to patients with newly diagnosed disease [26, 31].

There are many potential explanations for this association. One potential association is that this reflects confounding by cigarette smoking. That is, cigarette smoking is a well-known risk factor for RA [32] and is also a risk factor for PD [33], so the association between RA and PD could potentially reflect confounding by the shared risk factor of cigarette smoking. Arguing against this possibility is that the large studies discussed above took smoking into account, either through statistical adjustment [28] or by excluding smokers [29], yet the association holds. Furthermore, as discussed below, it is plausible that cigarette smoking is not simply a shared risk factor for PD and RA, but drives the increased risk of RA through the intermediary of PD.

Another potential mechanism accounting for the association between PD and RA is the possibility that oral microbiota might end up in the synovium, triggering a local inflammatory process. For example, Reichert et al. found genetic material from *P. gingivalis* in the synovium of 7/42 (16.7%) of RA patients vs 4/114 (3.5%) of HC, $p = 0.009$ [34]. However, this does not appear to be a specific finding, as similar organisms were also observed in the synovium of subjects with OA [35], and others have found that bacterial DNA as a whole is present in subjects with a variety of disorders [36–39]. It was suggested that this finding reflects non-specific trapping of killed bacteria by inflamed joints [40].

A third potential mechanism is that the arthritic process, and its therapy, may contribute to PD. That is, the immunosuppressive therapy of RA might predispose to the bacterial overgrowth that defines PD, or the decreased mobility of the hand and wrist resulting from the disease process in RA could impair oral hygiene, thus contributing to PD. This explanation would not entirely account for the findings of severe PD in patients with NORA [26, 31], nor for data showing that antibodies again *P. gingivalis* develop prior to the development of symptoms associated with RA [41]. More importantly, the possibility that active RA results in PD would not account for the findings reported in several prospective studies, in which periodontal therapy consisting of SRP has been shown to be therapeutic for RA [42–45]. In open-label studies, Erciyas et al. reported improved Disease Activity Score (DAS) levels and inflammatory markers among 60 subjects with mild or moderate RA who underwent SRP [42]; Biyikoglu et al. reported improved DAS and inflammatory markers among the 10 of 15 RA patients who underwent SRP and completed the study [43]; and Ribeiro et al. observed improved ESR among 22 subjects with RA who underwent SRP, but not in a parallel albeit not evidently randomized group of 16 subjects who underwent dental cleaning alone [44]. Although these open-label studies were not without biases in their design and analysis, similar findings were reported in a randomized study published by Ortiz et al. [45]. In this study, 40 subjects with active RA on stable therapy and severe PD were randomized to receive treatment for the latter versus no additional care, stratifying for baseline use of tumor necrosis factor inhibitor (TNFi) therapy in 20 of the subjects. They found substantial improvements in multiple clinical and laboratory markers of RA regardless of background TNFi use in the SRP arm.

A fourth potential mechanism accounting for the link between PD and RA is that as PD is an inflammatory process, PD and RA may reflect similar immunoregulatory environments that therefore might tend to co-occur in the same population, not unlike the associations between spondyloarthritis and inflammatory bowel disease or psoriasis. This possibility is supported by shared genetics between RA and PD, particularly among HLA-DRB1 alleles containing the SE [46]. There are multiple schema for classifying HLA-DRB1 risk alleles in RA but studies have largely shifted to analysis of amino acid residues rather than alleles. Amino acid residues encoded at positions 11, 71, and 74 in HLA-DRB1 are thought to be most important in RA risk [47]. To our knowledge, the association between PD and the SE at the amino acid level has yet to be

explored. Additional evidence for shared pathophysiologic mechanisms between RA and PD includes other genetic susceptibility factors [48], as well as findings that inflammation in periodontal tissue is mediated at least in part by cytokines such as interleukin (IL)-1, IL-6, and TNF, that have become therapeutic targets in RA [49]. However, the possibility that these shared mechanisms account for the association between PD and RA ultimately fail to account for the findings discussed above that treatment of PD results in improved clinical parameters in RA.

A fifth mechanism is that *P. gingivalis* may itself be the target of the immune system in RA. Several studies in patients with RA have shown elevated IgG antibodies directed against *P. gingivalis* [50–54]. However, other studies have reported contradictory findings [26, 55, 56], and the presence of these antibodies may reflect that this organism is present in the context of an inflammatory milieu without necessarily being pathogenic. Thus, although it is certainly plausible that there may be heterogeneity in the disease, with such antibodies contributing to the disease process in a subset of patients, the role of these antibodies in the pathogenesis of RA requires further study.

Finally, the association between PD and RA may be mediated by *P. gingivalis*, (the "2-hit" model of RA pathogenesis). According to this model, *P. gingivalis* contributes to RA through citrullination of proteins via its peptidylarginine deiminase (PAD) enzyme, resulting in the development of ACPAs [57]. ACPAs serve as diagnostic markers for RA, and third-generation ACPA assays have sensitivity ranging from 61.3 to 82.9 and specificity ranging from 93 to 97.6 for the diagnosis of RA [58]. Human proteins are not typically citrullinated. However, the PAD enzyme in humans and *P. gingivalis* converts the amino acid arginine into citrulline residues. Humans encode five PAD isotypes (PAD1-PAD4, and PAD6), of which PAD2 and PAD4 have been found in the synovial tissue and fluid of persons with RA, which may be a site where the citrullination occurs [59–61]. The significance of PAD in RA is underscored by studies showing that the PAD4 locus is associated with a ~ 2-fold risk of RA in a variety of populations [62–65]. *P. gingivalis* carries its own version of PAD (known as *P. gingivalis* PAD, or PPAD), possibly the only bacterial species that does so [66]. PPAD is capable of citrullinating human proteins [57]. There are several lines of evidence that this citrullination process may be directly pathogenic for the disease, rather than a bystander phenomenon. One is that in the collagen-induced arthritis model of RA, infection with *P. gingivalis* results in earlier onset and increased severity of the disease, findings that are abrogated if the *P. gingivalis* lacks PPAD [67]. Also, in the same model, tolerization with citrulline-containing peptides prior to induction of arthritis resulted in less disease severity and lower production of anti-CCP antibodies [68]. It is therefore of particular interest that cigarette smoking is associated only with anti-CCP+ RA [32], consistent with the possibility that cigarette smoking contributes to RA by inducing periodontitis. Of note, this association between *P. gingivalis* and RA may be limited to CCP+ disease, which is strongly associated with the major histocompatibility complex, particularly the SE [69, 70]. In contrast, *P. copri* appears to be more strongly linked to RA patients lacking the SE [11], who are often CCP-. *Thus, the pathophysiology of these two subsets of RA may be different, which clearly could have implications with respect to diagnosis and treatment*.

To summarize, multiple explanations for the association between PD and RA have been proposed. The model that arguably is best supported by the data is that PD is mediated in large part by a limited set of organisms, one of which is *P. gingivalis*. This species has the unique capacity to citrullinate human proteins, which when modified are targeted by the immune system to form ACPAs, the hallmark antibody of RA. Cigarette smoking may play into this association largely by increasing the risk of PD, thus accounting for its association with anti-CCP+, but not anti-CCP-, RA. The most important clinical implication of this theory is that treatment of PD appears to result in improvement in the RA disease process. This model is shown in Fig. 15.1.

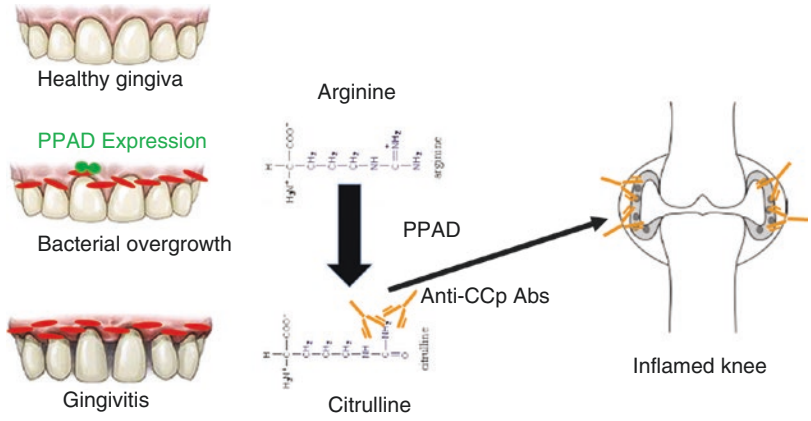

Fig. 15.1 Overgrowth of pathogenic bacteria such as *P. gingivalis* (red) occurs in the gingiva, resulting in inflamed tissue. This bacterial overgrowth is associated with expression of PPAD, which converts the amino acid arginine into citrulline. Antibodies against citrulline (anti-CCPs) then deposit in synovial tissue, resulting in arthritis

Additional Microbiomes in RA

As detailed above, much of the literature on the microbiota in RA has centered on the enteric or gingival microbiota. One other habitat that may be relevant is the lung. Interstitial lung disease is common in RA patients [71], indicating that the lungs may be a source of inflammation. Perhaps due to relative inaccessibility, the microbiota of the lungs has not been studied extensively. Recently, Scher and colleagues performed bronchial alveolar lavage on 20 patients with NORA, 12 healthy controls, and 10 patients with sarcoidosis [72]. The RA patients demonstrated decreased alpha diversity and depletion of several families, such as Burkholderiaceae, Actinomycetaceae, and Spirochaetaceae. However, similar findings were seen in the patients with sarcoidosis, and principal coordinates analysis showed that the sarcoidosis and RA patients clustered together, apart from the controls. Thus, Scher concluded that these findings may reflect an inflammatory lung, rather than a specific RA, phenotype. This stands in contrast to the gut microbiota studies, where several of the findings—particularly the outgrowth of *P. copri*—appear to be unique to RA [11].

One final habitat that was evaluated in a single study is the salivary microbiota. Note that these results cannot be compared with those of the gingival microbiota, as these are two fairly distinct habitats [73]. Counterintuitively, Zhang et al. found *P. gingivalis* among multiple other organisms to be depleted in the saliva of NORA patients, while several species of *Prevotella* were elevated in the RA saliva [15]. Interestingly, the same study also found several species of *Prevotella* to be elevated in the control gingival plaques. Partial normalization of the oral microbiota was observed following introduction of immunosuppressive therapy.

Therapeutic Alterations of the Microbiota

Antibiotics

There have been numerous controlled studies of antibiotics as potential therapeutic agents in RA. As summarized in Table 15.1, this benefit was seen in multiple different classes of antibiotics, including fluoroquinolones, tetracyclines (minocycline > doxycycline, tetracycline), and sulfa antibiotics, including but not limited to sulfasalazine. The effectiveness of antibiotics may not necessarily be attributable to their antimicrobial activity, as many of them particularly the tetracyclines may contain intrinsic anti-inflammatory activity, such as inhibition of matrix metalloproteinases (MMPs) [74]. Indeed,

O'Dell and colleagues suggested that the effectiveness of low-dose doxycycline in one study proved that the mechanism was through inhibition of MMPs [75], although none of these studies included an assessment of the microbiota. It has also been proposed that the effectiveness of tetracyclines and sulfa drugs is due to their ability to eradicate oral pathogens [40]. As we learn more about potential microbial contributing factors to RA, the possibility that antibiotics were effective due at least in part to antimicrobial activity becomes increasingly plausible. Whatever the mechanism of effectiveness, antibiotics are generally not considered optimal long-term therapy, due to risks such as resistance to antibiotics and development of *Clostridium difficile* colitis. The key perhaps is to find means of altering the microbiota that do not carry the risks associated with antibiotics. For example, as discussed above, specific therapy of periodontal disease appears to be effective therapy for RA [45] perhaps by eradicating *P. gingivalis*, an approach that has a better safety profile than long-term use of antibiotics.

Probiotics

There have been four small sample size RCTs of probiotics in RA (Table 15.3). A fifth study was published [76] but appears to be duplicative of one of the other four [77]. Although two of them reported positive findings, these effects were minimal. For example, Mandel et al. [78] reported efficacy on the basis of small effect sizes and non-statistically significant findings such as improved patient-reported ability to participate in daily activities in 4/22 (18%) in the probiotic arm as compared to 2/22 (9.1%) in the placebo arm, $p = 0.53$. Likewise, after excluding 14 of 60 subjects from the analysis due to failure to follow the protocol, Alipour et al. [77] reported that the swollen joint count decreased from a mean (25th–75th percentiles) of 0 (0–2) to 0 (0–1) in the intervention group, compared to a decrease in the placebo group from 1 (0–1.75) to 1 (0–1.75); the between-group p-value was not reported. They did, however, report that patients given the probiotic were more likely to have a EULAR response (8/22 [36%] vs 1/24 [4.2%], $p = 0.007$) as well as significantly lower inflammatory cytokine levels in the probiotic group. Overall, however, the effects of probiotics in RA appear to be small at best. As will be discussed in the individualized medicine chapter (Chap. 35), there are multiple reasons for this lack of substantial effects, including failure of the probiotic to alter the microbiota or the selection of the wrong probiotic.

Diet

Although dietary therapies can rapidly alter the microbiota [79, 80] dietary therapy has also not been found to be a successful therapeutic approach for RA. A Cochrane review evaluated

Table 15.3 Probiotic trials in RA

Study	*n*	Probiotic	Duration	Outcome
Hatakka et al. [110]	21	LGG	12 months	No statistically significant differences in a variety of clinical and immunologic parameters
Mandel et al. [78]	45	BC	60 days	ACR20 attained by 8/22 (36%) completers of BC vs 6/22 (27%) completers of placebo, *p*-value not provided. No statistically significant differences in a variety of patient-reported outcomes or laboratory values
Pineda et al. [111]	29	LR	90 days	ACR20 attained by 3/15 (20%) probiotic vs 1/14 (14%) placebo ($p = 0.33$)
Alipour et al. [77]	60[a]	LC	8 weeks	Very minimal improvements favoring probiotic

[a]Out of 60 initial participants, only 46 were analyzed; the other 14 were excluded due to not following the protocol
BC Bacillus coagulans, *LC Lactobacillus casei*, *LGG Lactobacillus rhamnosus* GG, *LR Lactobacillus reuteri* RC-14 and *Lactobacillus rhamnosus* GR-1 (both administered)

15 controlled dietary intervention studies in RA, including vegetarian, elemental, vegan, and Mediterranean diets [81]. There were no consistent benefits observed. The vegetarian and Mediterranean diets resulted in decreased pain, but no improvements in function or objective findings. Other interventions likewise failed to show substantial effects. The authors also noted substantial dropout in the treatment arms, which they attributed to diet unpalatability. The failure of some of these studies may pertain to some of them evidently being carbohydrate-rich diets, which might have the effect of increasing the abundance of *Prevotella* [79], which as noted above may not be optimal in patients with RA. Clearly, future interventions must be targeted towards eradicating known dysbiosis. An additional factor to consider is that a patient's baseline microbiota may influence response to dietary therapy [82] and thus may need to be assessed as part of the intervention.

Concluding Remarks

Over 100 years ago, RA was considered to be an infectious disease. By the late twentieth century, this hypothesis had fallen out of favor, even though some clinical trials of antibiotics showed effectiveness. Currently, there is accumulating evidence that there are infectious triggers to RA. That two geographically distinct studies of newly diagnosed subjects with RA have both shown an abundance of *P. copri* in their intestines [11, 13], particularly in light of the data showing the same organism to be an immunologic target in RA [21], is highly suggestive that this organism may be part of the pathogenesis of the disease. Given that its abundance appears to be normal in established disease [11, 14], it remains to be seen whether attempts to target this organism therapeutically might bear fruit. In contrast, the gingival microbiota, particularly in patients with severe periodontal disease, appears to be a worthwhile therapeutic target. Whether antibiotics are effective due to their ability to eradicate oral pathogens is unclear, and few would advocate chronic use of antibiotics in light of the array of medications available today. However, just as routine screening of the eyes is part of the management of children with JIA, perhaps routine screening of the gingiva should be part of the care of RA patients, with appropriate local therapy as needed.

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