Gene/Environment Interaction and Autoimmune Disease

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Abstract

Autoimmune diseases are complex illnesses in which the body's immune system attacks its own healthy tissues. These diseases, which can be fatal, gravely impact the quality of life of those afflicted by them with no cure currently available. The exact etiology of autoimmune diseases is not completely clear. Biomedical research has revealed that both genetic and environmental factors contribute to the development and progression of these diseases. Nevertheless, genetic and environmental factors alone cannot explain a large proportion of cases, leading to the possibility that the two factors interact in driving disease onset. Understanding how genetic and environmental factor influence host physiology in a manner that leads to the development of autoimmune diseases can reveal the mechanisms by which these diseases manifest, and bring us closer to finding a cure for them. In this chapter, we will review the current research of genetic/environmental interactions that contribute to development of autoimmune diseases, with an emphasis on interactions between the host and the multitudes of microbes that inhabit it, the microbiota.

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1) Introduction

What are autoimmune diseases

Autoimmune diseases are complex illnesses in which the body's own immune system attacks and destroys the body's own healthy tissues. Many tissues and organs can be affected by autoimmune diseases such as the skin, joints, intestines, endocrine glands (thyroid, pancreas, etc.) and blood vessels[1]. Over 80 different diseases have been recognized as autoimmune diseases and as a group they affect more than 8% of the world population[2]. Symptoms of autoimmune diseases can range from fatigue and malaise to life threatening organ failure. Although decades of research was dedicated to understanding the cause and course of these diseases, we still do not fully understand why they develop or how to cure them[3].

At the core of all autoimmune diseases is an improper response of the immune system against the body's healthy tissues. The human immune system is an immensely powerful cellular weapon, designed to attack invaders it deems as foreign, or non-self (not an integral part of the body). Many cellular regulatory processes are in place to prevent the immune system from recognizing its own body as an invader. For example, T and B cells that are found to be reactive to self-antigens are destroyed or are put in a state of anergy (immunologically non-functional) before becoming active. This recognition of the body as "self" is called self-tolerance, and malfunction of this process is at the heart of autoimmune diseases[1].

Autoimmune diseases are both chronic in nature, and currently incurable. As such, they pose a major burden on healthcare systems while causing significant individual suffering. Current treatments focus on relieving symptoms, and since autoimmune diseases are caused by faulty immune system function, these treatments attempt to suppress immune function in the patients. This leaves the patients susceptible to infections and cancer development. As with other incurable

diseases, a major focus of research has been on identifying the causes of autoimmune diseases with the goal of preventing them. So far, several inheritable genetic factors were identified which explain some autoimmune diseases, while several environmental factors were found to explain others. Still, the cause for most autoimmune disease cases are unknown, leading to the notion that interactions of genetics and environmental factors are responsible for disease onset[3].

Genetic factors associated with autoimmune diseases

The observation that most autoimmune diseases feature familial clustering has led to the notion that genetic risk factors might be involved in development of autoimmune diseases. Advances in DNA sequencing technologies in the past century has provided much needed insight into these genetic risk factors. Indeed, genome wide association studies (GWAS) have identified that certain genetic variants are shared across multiple autoimmune diseases, suggesting the certain shared pathways are dysregulated in these diseases. The most common genetic risk factors are variants in the HLA locus, which enables recognition of many different foreign antigens, but can also impact recognition of self-antigens[1]. Also worth noting are variants in STAT4 and IL-23R, which have a central role in regulating the adaptive immune response, and are shared across many different autoimmune diseases[4,5].

Although GWAS studied were a source for much hope, they still cannot explain most autoimmune diseases cases. Reflective of this is the finding that concordance rates of autoimmune diseases in monozygotic twins ranges between 12% for certain autoimmune diseases and 67% for others. Thus, attention has shifted to uncover environmental factors that might explain the gap between identification of disease-associated genetic variants and disease occurrence[3].

Environmental factors associated with autoimmune diseases

Numerous studies have established a connection between exposure to environmental factors and the development of autoimmune diseases. Indeed, the observation that geographical location and individual lifestyle choices can affect autoimmune diseases development rates supports this. These environmental factors may be physical, such as UV radiation; chemical, such as exposure to pesticides or tobacco smoke; or biological, such as infection by pathogenic microbes[2]. Another biological factor that has recently come into the spotlight is the human microbiome[6].

The microbiome

Humans, like most other animals, are colonized by a multitude of microorganisms. These include archaea, bacteria, fungi, viruses and protozoa, which are collectively termed the microbiota. The microbiota of a human adult accounts for 1-2 kg of total body weight and are spread out across many tissues such as skin, urogenital tract, and respiratory tract, with the largest community of microbes residing in the gastrointestinal tract. The genomic data the microbiota contains, and thus their ability to produce proteins which affect human physiology, is collectively termed the microbiome[6].

Most members of the human microbiota require very specific growth conditions. Thus, our understand of the function of the microbiota was limited to the species that we could culture in the lab. Advances in sequencing technology now allows us to identify most members of the human microbiota, and to infer their potential for influencing human physiology from their genome[6]. Additionally, use of germ-free mice (sterile mice reared in isolators which allow mono-colonization with a single species of bacteria or whole microbiota transfer from a human donor)

has allowed us to move from correlative studies to more mechanistic studies. Now, we can identify each member of the microbiota and the proteins they produce, colonize germ-free mice carrying the genetic background of choice with these microbes, and discover how genetics and environmental factors interact in the development of many diseases[6].

In the past 15 years or so, the microbiota was found to influence almost every facet of human physiology. For example, the microbiota has been found to contribute to obesity and metabolic syndrome[6]. Recent studies have linked microbiota composition and metabolic activity to neurodegenerative diseases like Alzheimer's and Parkinson's disease[7] and other neurological conditions such as autism[8] and psychosis[9]. Most relevant, the microbiota was found to have a major role in shaping the immune system. It is now though that the microbiota "educates" the immune system, helping it distinguish symbiotic microbes from pathogenic ones. In the context of autoimmune diseases, the microbiota was found to trigger immune regulatory factors such as maturation of T regulatory cells and secretion of anti-inflammatory cytokines. This is achieved by metabolizing nutrients into short-chain fatty acids (SCFA) which regulate the innate and adaptive immune response, and activation of pattern recognition receptors such as toll-like receptors and Nod-like receptors, amongst others[6]. The factors controlling microbiota composition are varied and are still being extensively studied. While genetics have a role in shaping microbiota composition, the most influential factors seem to be environmental such as diet, use of medicine and geography[10].

2) Gene/Environment Interaction and Autoimmune Disease

2.1) Inflammatory bowel diseases

Inflammatory bowel diseases (IBD) are a group of chronic inflammatory disease affecting the gastrointestinal tract with unknown etiology and no cure, disturbing the lives of tens of millions across the world[11]. The two major types of IBD are Crohn's disease and ulcerative colitis. Crohn's disease is characterized by transmural inflammation that can manifest anywhere along the gastrointestinal tract, from mouth the anus, though most cases are confined to the last segment of the small intestine, the ilium. Ulcerative colitis on the other hand is confined to the terminal segment of the gastrointestinal tract, the colon, and is characterized by ulceration of the colonic mucosa. Both diseases appear in episodes of flares followed by a remission period. These diseases have a massive effect on morbidity and mortality and left untreated could develop to bowel cancer. Current treatment options include agents that suppress the immune system such as steroids and antibodies targeting cytokines, while last resort treatments are resection of inflamed sections of the intestine[12].

The pathogenesis of IBD includes some features of autoimmune disease, such as the presence of autoantibodies, but also features of immune-mediated diseases, such as dysregulation of cellular immunity and exaggerated response to luminal content. This exaggerated response of the immune system does not seem to target a certain member of the microbiota, which is evident by the fact that antibiotic treatment holds little therapeutic effect in IBD. While IBD are not characterized by auto-reactive T cells, which is one of the postulates of autoimmune diseases, transfer of T cells in animal models can still transmit IBD-like conditions between hosts, which is another postulate. Thus, even though not a "classic" example of autoimmune disease, IBD are still considered as such, a classification which affects treatment routes[13,14].

The etiology of IBD are currently unclear, though research points to an interaction of host genetics and environmental factors. GWAS studies have identified about 200 IBD susceptibility

genes. The most prevalent mutations discovered to be associated with IBD are in genes involved in innate and adaptive immune responses, intestinal barrier function and autophagy. However, these can explain only a small percentage of IBD cases. Additionally, study of monozygotic twins has shown only low concordance rates for development of IBD[15]. Strikingly, studies following immigration of Asian population to America and Europe has found a sharp increase in the incidence of IBD in first and second-generation immigrants[16–18]. This, and the rise of disease prevalence in industrialized countries in the 20th century, has led to the hypothesis that environmental factors might also be involved in development of IBD[19].

Gene-cigarette smoke interaction in IBD

One of the most studied and reliably reproducible environmental factors affecting IBD risk is cigarette smoking. While smoking cigarettes significantly elevates the risk of developing Crohn's disease, it has a surprising protective effect on development of ulcerative colitis, with non-smokers having a 4-fold higher risk of developing ulcerative colitis compared to smokers[20]. Currently, the exact mechanism conferring disease susceptibility or resistance from cigarette smoking is not clear.

Several IBD risk associated genes have been shown to interact with cigarette smoking in affecting the risk of IBD development. An increased risk for development of Crohn's disease has been found in cigarette smokers harboring a mutation in the CYP2A6 gene, which encodes an enzyme involved in metabolism of nicotine. The same study also demonstrated that smoking cessation is associated with an increased risk of developing ulcerative colitis, but only in patients carrying a mutation in the GSTP1 gene which encodes a glutathione transferase protein[21]. A different study has used the method of logic regression to show that cigarette smoker carrying a

mutation in the gene CALM3, a calcium-binding protein that affects different kinases, were three times less likely to develop ulcerative colitis compared to non-smokers carrying the same mutation[22]. Another study has identified that mutations in the IL-23R gene, which encodes for an immune related receptor previously recognized as a risk factor for development of Crohn's disease, interact with cigarette smoking to dramatically increase disease risk[23].

So far, mechanistic studies linking genetic predisposition to cigarette smoke in IBD development have been few. A large multi-center study examining about 20,000 IBD patients has discovered 64 SNPs to be associated with altered IBD risk in cigarette smokers compared to non-smokers. Most of the identified SNPs affected genes involved in immune and barrier function. They went on to demonstrate that genetic deletion in mice of two of the identified SNPs (IL-10 and NOD2) increased the animals' susceptibility to colitis after exposure to cigarette smoke, thus validating their findings in humans[24]. A different group has built on previously reported data that mutation in an autophagy gene, ATG16L1, interacts with cigarette smoking to elevate risk of developing Crohn's disease. Looking at both Crohn's disease patients and mutant mice, they show that cigarette smoke disrupts the antimicrobial function of Paneth cells, specialized secretory cells in the intestine, but only in patients and mice carrying the mutation in ATG16L1[25]. Mechanistic works such as these will likely expand in the future to explain how cigarette smoke interacts with host genetics and help identify new prevention and treatment strategies for IBD.

Gene-microbe interaction in IBD

The microbiota of IBD patients has been extensively studied in the past decade and has clearly demonstrated that the microbiota of IBD patients is fundamentally different than healthy controls[26]. While massive amounts of data have been generated, it still isn't clear whether this shift

in microbiota composition (termed dysbiosis) precedes manifestation of these diseases and perhaps drives them, or whether this dysbiosis is the result of the chronic inflammation. Recent works have revealed that the conditions created in the gut by the inflammatory state is actually favorable for expansion of virulent bacteria and might explain the repetition of flares and remissions observed in these diseases[27]. Currently, fecal microbiota transfer has not been shown to be efficient at treating IBD[28], though it has been shown to be feasible in animal studies[29] and in other diseases such as *Clostridium difficile* infection[30]. Yet, identification of a certain composition and structure of the gut microbiota which would allow early detection and prevention of IBD has not been revealed.

Given the genetic background of IBD (and the fact that it explains only low amounts of cases), the realization that environmental factors contribute to disease risk[31], and the effect host genetics has on the microbiome[32], focus has shifted to gene-microbiota interaction to try and explain most cases of IBD[33]. Experiments in genetically altered mice have shown that mutations in certain IBD risk genes can lead to dysbiosis of the gut microbiota of these animals[34,35]. However, transferring this microbiota to germ-free mice does not lead to IBD-like pathology, as happens with mice carrying non-IBD related mutations (T-bet$^{-/-}$, Rag2$^{-/-}$, TLR5$^{-/-}$ and NLRP6$^{-/-}$)[36–38]. Since mice with mutations in the most prevalent IBD associated gene (NOD2, ATG16L1 and IRGM) have been shown to have impaired pathogen clearance, it is possible that host mutations which drive dysbiosis might also make the host more susceptible to infection by these dysbiotic microbes.

While no specific pathogenic bacteria have been associated with all IBD cases, one such microbe, adherent-invasive *Escherichia coli* (AIEC), was found to be associated with many cases of IBD[39]. Interestingly, these associations seem to be dependent upon host genetics, thus forming a gene-microbe interaction. AIEC have the ability to attach to, and invade intestinal epithelial cells,

triggering disease. AIEC attaches to intestinal epithelial cells by binding to host protein CEACAM6, which is not expressed in healthy tissue, but only in inflamed epithelial cells[40]. CD patients with ileal disease were found to abnormally have AIEC attached to their intestinal epithelium, and AIEC numbers seems to correlate with disease severity[41]. A Study has found that epithelial cells lacking IBD risk genes NOD2, ATG16L1 and IRGM were not able to clear the invading AIEC[42]. Additionally, while mice are not susceptible to AIEC colonization of the intestine, mice lacking the NOD2 gene display high numbers of infiltrating AIEC in intestinal lymph nodes[43]. Thus, it seems that in individuals carrying genetic risk factors these bacteria take advantage of host susceptibility to invade, and might be a factor contributing to disease development[44].

As in the above example, it is possible that certain microbes take advantage of host genetic susceptibility to invade and trigger disease which might progress to an IBD. For example, mice carrying a mutation in the Crohn's disease risk gene *ATG16L1* were shown to be highly susceptible to infection by an invasive foodborne pathogen[45,46]. Another possibility is that infection of an individual carrying mutations in IBD risk genes will enhance disease susceptibility. This was shown with mice lacking ATG16L1 that were infected with the foodborne pathogen norovirus. When these mice were infected with the virus, they displayed reduced antimicrobial protein secretion by intestinal goblet cells which left them susceptible to development of colitis in a chemical model[47].

To summarize, mutations in IBD risk genes in the host can affect microbiota composition and susceptibility to infection by various pathogens. This can then leave the genetically predisposed host in risk of developing a chronic inflammation in the bowel as is seen in IBD.

2.2) Psoriasis

Psoriasis is an immune-mediated systemic disease that manifests on the skin as well defined thick red plaques with overlying silver scale[48–50]. Psoriasis affects an estimated 2-3% of the population of the western world making it a common autoimmune disease[48,51,52]. In the majority of cases, psoriasis is limited to the skin, but anywhere between 2-10% of psoriasis patients also develop associated inflammation and destructions of the joints, termed psoriatic arthritis[48,51,53].

Though originally thought of as a merely cosmetic affliction with limited systemic health implications, it is now known that psoriasis is a systemic condition, with a range of comorbidities[54]. Psoriasis patients develop Crohn's disease at a higher rate than the general population and have a higher incidence of psychiatric disorders and uveitis[51,55,56]. In the last ten years it has also been established by large population studies that psoriasis is associated with metabolic syndrome and is an independent risk factor for cardiovascular disease[57–59].

As is the case for IBD, the classification of psoriasis as an autoimmune condition is controversial. There is little understanding of what triggers initial diseases presentation and no clearly identified self-antigen. It is also suspected that the breakdown in immune tolerance in psoriasis likely happens in the innate immune system[60]. Despite the limits in our understanding of the pathophysiology of psoriasis, there is a consensus that immune dysregulation characterized by aberrant activation of Th1 and Th17 immunity and high levels of TNF-α mediate the skin findings in psoriasis[60]. The key role of T-cells and their cytokine profile in the pathophysiology of psoriasis is highlighted by the advent of anti-TNFα, anti-IL-23, and anti-IL-17 monoclonal antibody therapeutics, which are effective in treating psoriasis and leading to disease control[48,61–63].

Genetics

Psoriasis has been shown to have a strong genetic component, with a higher concordance of psoriasis between monozygotic twins compared to the concordance between dizygotic twins. Cases of psoriasis also cluster within families[64]. Numerous GWAS studies have been completed on families with a predisposition to psoriasis[65–67]. In gene-linkage studies the MHC class I region has the strongest association with psoriasis; with the HLA-Cw*0602 allele being implicated in more than half of patients with plaque-type psoriasis[60,64,68,69]. In keeping with the known role that T-cell subsets play in the pathophysiology of psoriasis, genes that are known to regulate T-cell function, such as *IL23R*, *IL12B*, *IL23A*, *TRAF3IP2*, *RUNX3*, *TAGAP*, and *STAT3*[5,70], have all been implicated in GWAS studies. In addition, recent work has identified susceptibility loci that link genetic alterations in the innate immune system with psoriasis. Genes identified in these studies play a role in macrophage activation, NF-κB signaling, and interferon-mediated antiviral responses[70]. Several of the identified loci have also been associated with Crohn's disease, ankylosing spondylitis and celiac disease, strengthening the hypothesis that these autoimmune conditions might have a shared etiology[5].

Environment

It is clear that genetics alone does not explain the presentation of psoriasis. Even though disease onset and severity are similar for monozygotic twins, concordance between monozygotic twins is not 100%[71]. Furthermore, the identified MHC class I susceptibility loci are seen in only half of patients with psoriasis[64]. Taken together, these findings highlight that environmental factors also play a role in the pathogenesis of psoriasis.

Ultra-violet (UV) light is thought to modify psoriatic disease. Though, there is little correlation between latitude and the prevalence of psoriasis in a given region, ultraviolet light

exposure is known to improve psoriasis and both psoriasis and psoriatic arthritis improve in the summer months[49,71].

Several studies have also shown a strong association between psoriasis and people who currently or previously smoked cigarettes. Cigarette smoking increases the risk of psoriasis in a dose-dependent manner and smoking has been shown to effect response rates to therapy[72,73]. Amplifying this observed link have been studies that have specifically looked at the combined effect of carrying a genetic susceptibility allele and smoking. Two separate studies looked at smokers that carry the HLA-Cw6 allele and genetic variants at the *CSMD1* and the *TNIP/ANXA6* loci. In both studies, people that smoke and carry these genetic variations showed an increased risk of developing psoriasis compared to non-smokers. For HLA-Cw6, the psoriasis risk was greater than 10-fold for smokers that carried the allele compared to non-smokers without the allele[74,75].

The microbiota has also been shown to be modulated in psoriasis. In pediatric populations the onset of a specific clinical presentation of psoriasis called guttate psoriasis, with small rain-drop sized psoriatic plaques, has been specifically associated with *Streptococcus pyogenes* (*S. pyogenes)* infections of the skin and of the pharynx[76,77]. *S. pyogenes* infection has also been shown to exacerbate the more common variant of psoriasis, plaque psoriasis[78]. The T-cells of patients with psoriasis and *S. pyogenes* pharyngeal infections recognize M-proteins expressed by *S. pyogenes.* These M-protein specific T-cells show increased expression of skin homing molecules and cross-reactivity with skin antigens. Additionally, in a single prospective study, tonsillectomy led to a reduction in the circulating M-protein specific skin homing T-cells and decreased the severity of psoriasis[79,80]. In sum, these finding suggest that *S. pyogenes* infection may play a role in the both disease initiation and propagation of psoriasis[81].

With the advent of next generation sequencing, recent studies have looked more broadly at the links between the microbiome and psoriasis. The impetus for these studies was the observation that Crohn's disease patients have a 7-times higher risk of developing psoriasis than the general population[54]. As evidence mounts that imbalances in the gut microbiome may trigger immune-mediated inflammatory disorders such as Crohn's diseases, investigators began to postulate that imbalances in the gut microbiome might also be associated with psoriasis and psoriatic arthritis[82]. An initial study used PCR to ask specifically if patients with psoriasis showed a depletion of *Faecalibacterium prausnitzii* and an increase of *Escherichia coli*- gut microbiome shifts that had been previously reported in patients with inflammatory bowel disease[83]. This report did show similar shifts in patients with psoriasis, though almost half of the psoriasis patients that were studied also had IBD[84]. A second study, which looked more globally at bacterial communities in psoriasis using 16*S* sequencing, revealed a decrease in the diversity of the gut microbiome in patients with psoriasis and psoriatic arthritis compared to health controls, with specific reductions in the taxa *Akkermansia*, *Ruminococcus*, and *Pseudobutyrivibrio*[85]. A more recent study attempted to characterize the skin microbiome in 52 patients with active psoriasis with comparison to the data of 300 healthy individuals in the NIH human microbiome studies. This study found the opposite of the first, with increased diversity of the gut microbiome in patients with psoriasis compared to controls. Increases in *Akkermansia, Ruminococcus*, and *Faecalibacterium* and decreases in *Bacteroides* were also observed[86]. The differences in these study findings likely reflects the current lack of standardization in the field, as Scher et al. completed 16*S* sequencing with sampling of the V1-V2 region compared to the use of V3-V4 sequencing used by Codoñer et al. Though the shifts are different, the studies conducted on the gut microbiome do show that patients with psoriasis have a different microbiome than patients without psoriasis. These findings

are significant, as in mouse models of psoriasis mice that lack a microbiota showed a lower degree of local and systemic Th17 inflammation[87].

The skin is also home to a diverse population of bacteria[88,89] that might function in the pathogenesis of psoriasis. The composition of the skin microbiome is site specific, with oily, wet, and dry locations of the body creating unique ecological niches with distinct microbial communities[89–91]. In a highly standardized study sampling six body sites in both psoriasis patients and controls, 16*S* sequencing showed higher diversity in the psoriasis-associated skin microbiome; with enrichment of *Staphylococcus aureus* and decreased abundance of *Staphylococcus epidermidis* and *Propionibacterium acnes*[92]. Smaller studies also support an increased abundance of *Staphylococcus aureus* and a decrease in the abundance of *Propionibacterium acnes*[93,94]. It is still unclear whether shifts in the skin microbiome drive disease progression in psoriasis. There is some evidence that the colonization of neonatal mice with *Staphylococcus aureus* skews the immune system towards Th17 immunity, which would support the hypothesis that the increase in *Staphylococcus aureus* seen in these studies could drive the inflammation observed in psoriasis[92]. However, it is still possible that the changes observed in the skin microbiome of psoriatic plaques are secondary to the dry inflamed environment, rich in antimicrobial proteins, created by the underlying disease[50]. Additional, work will need to be completed to define an etiological function for the skin microbiome in psoriasis.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune condition affecting 0.5-1% of the world population [95]. RA often presents clinically with symmetrical inflammation of the small joints of the hands, progressing with time to a destructive and debilitating systemic arthritis. In RA, a

breakdown in immune tolerance in the adaptive immune system leads to the develop of characteristic auto antibodies, rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). RF and ACPA are associated with joint inflammation and also cause inflammation of the heart and lungs, further contributing to the increased mortality seen in this condition [96].

RA is more common in women and in people with a genetic susceptibility to the disease [97]. The allele of greatest significance is HLA-DRB1, which encodes an MHC class II molecule. HLA-DRB1 mutations are seen in more than half of patients with rheumatoid arthritis and have been specifically linked to patients who develop the auto antibodies RF and ACPA. Several different alleles of HLA-DRB1 have been linked to RA. Though these alleles vary, they all share an amino acid sequence in a single hypervariable domain. The hypervariable domains are the regions of the MHC class II molecules involved in antigen recognition. Thus, in the current paradigm, HLA-DRB1 alleles associated with RA are capable of generating an immune response to unique self-antigens and initiating the breakdown in adaptive immunity seen in this condition [96,98–100]. Mutations in *PTPN22*, *CTLA4*, *TRAF1/C5* region, and *c-REL* have also been associated with the development of rheumatoid arthritis [101–104]

Environment

Smoking

The low 15-30% concordance rate in RA between monozygotic twins indicates that environmental triggers also play a role in the pathogenesis of RA [105]. As has been observed in other autoimmune diseases, smoking greatly increases the risk of developing rheumatoid arthritis. In the case of rheumatoid arthritis, the mechanism linking smoking to disease is more established. Patients with rheumatoid arthritis that harbor HLR-DR4 susceptibility alleles and smoke, develop

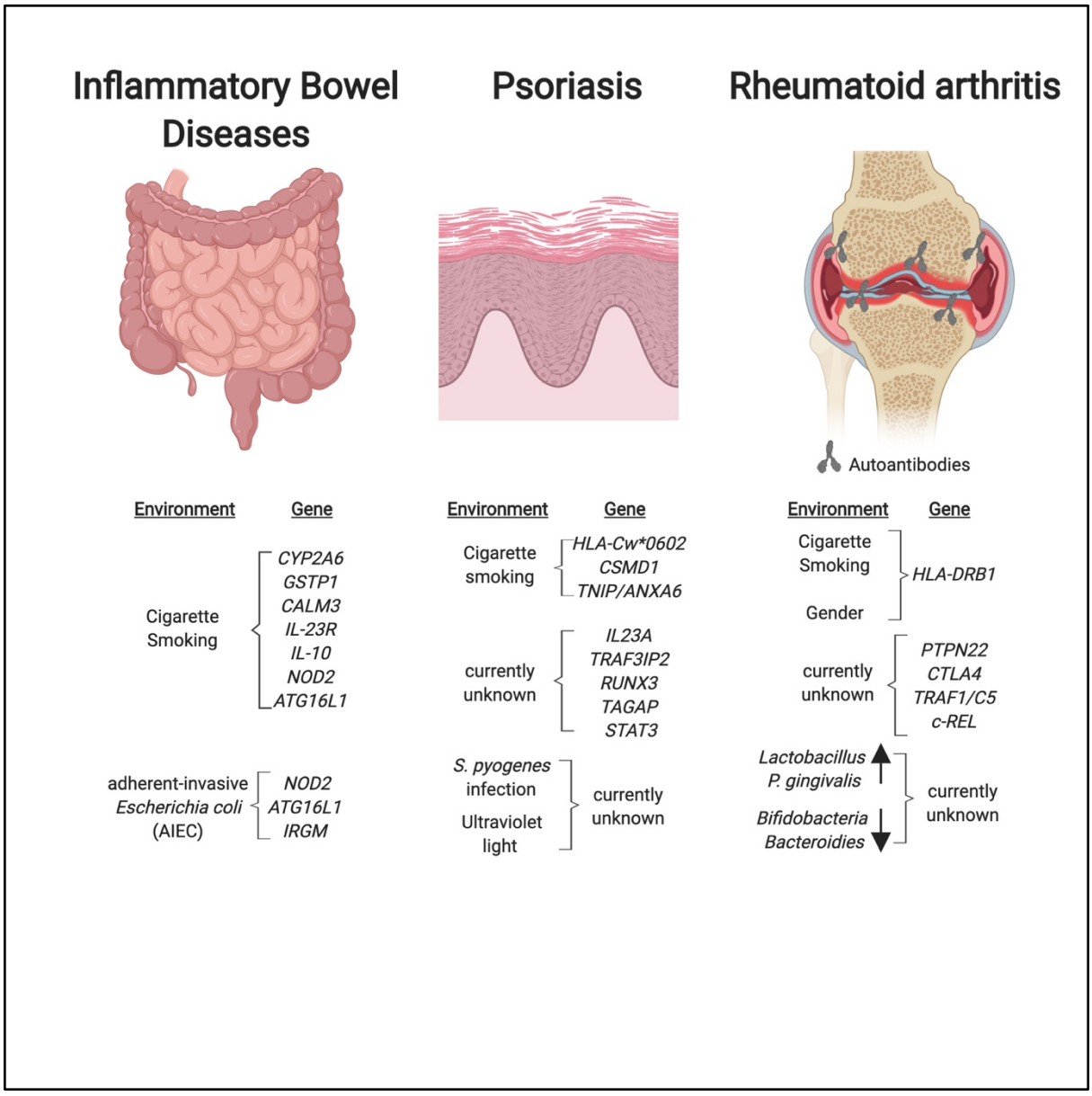
a serological subtype of RA characterized by RF and ACPA [106]. ACPA, anti-citrullinated protein antibodies, are thought to develop in response to host proteins that undergo the post-translational modification process known as citrullination. Citrullination is catalyzed by peptidylarginine deiminase enzymes, which convert arginine within host proteins to citrullines. These citrullinated proteins act as neo-antigens. People with HLA-DR4 susceptibility alleles have immune systems that are primed to recognize citrullinated host proteins and thus develop an immune response that triggers the inflammation and auto-antibody profile seen in RA. Smoking has been shown to increase the activity of peptidylarginine deiminases and therefore initiates the first steps in the breakdown of the adaptive immune system [107]. These finding, linking specific alleles and smoking to the subsequent development of rheumatoid arthritis, have been shown and confirmed in numerous large population studies (reviewed by [108]).

Infection and the Microbiome

The first observations linking the gut microbiota to RA were in the 1960s with the finding that patients with arthritis showed and expansion in *Clostridium perfingens* type A [109]. Though this organism was not specifically linked to RA in future studies, the concept that the gut microbiota can modulate arthritic inflammation was subsequently confirmed in both mouse and human studies. As early as the late 1970s, it was shown that rats raised in a conventional facility, or colonized with *E.coli* and *Bacteroidies* were protected from developing inflammatory arthritis in a rat adjuvant arthritis model, when compared to germ free rats raised without the beneficial effects of the microbiota [110–113]. Furthermore, several genetic models that induce spontaneous inflammatory arthritis in mice have been shown to be microbiota dependent, solidifying the interplay between genetic susceptibility and the microbiome in the pathogenesis of inflammatory

arthritis[114,115]. In humans, 16*S* and metagenomic studies comparing the microbiomes of patients with RA to unrelated controls show decreased enrichment of the beneficial microbes *Bifidobacteria* and *Bacteroidies* [116,117]. Two separate studies also showed an expansion in *Lactobacillus* in the microbiome of patients with RA compared to controls [118,119]. Prior infections with Epstein bar virus and parvovirus B-19 have also been associated with RA in small studies, with higher titers of anti-viral antibodies in patients with RA compared to controls [120–122]. Taken together, these data establish a role for dysbiosis or antecedent infection in the pathogenesis of RA.

The oral microbiome has also been linked to RA. Early in disease, patients with RA have a higher incidence of periodontal disease with twice the carriage rates of *Porphyromonas gingivalis (P. gingivalis)* compared to people without RA [123–125]. Interestingly, *P. gingivalis* express the enzyme peptidylarginine deiminase. As described above for cigarette smoking, peptidylarginine deiminases catalyze the conversion of arginine residues within host proteins to citrullines, triggering the production of anti-citrullinated antibodies in genetically susceptible individuals. Thus, oral dysbiosis might be an initiating event in the pathogenesis of RA [126–128].



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