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Current concepts on the immunopathogenesis of inflammatory bowel disease

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1. ABSTRACT

Inflammatory bowel disease (IBD) is comprised of both ulcerative colitis (UC) and Crohn's disease (CD) and is a chronic inflammatory disorder that results from a dysregulated immune response in genetically susceptible individuals. Data from genome-wide association studies (GWAS) have identified approximately 70 genetic loci that confer susceptibility to CD and over 30 loci that are associated with UC. These genetic loci guide the understanding of the molecular mechanisms of these gene products and reveal that alterations in the immune response underlie the pathophysiology of IBD. This review highlights critical areas in the microbiome, innate immune response, and the adaptive immune response that lead to the chronic mucosal inflammation typically seen in IBD.

2. INTRODUCTION

IBD affects approximately 1.4 million Americans and comprises two complex, relapsing diseases characterized by chronic inflammation of the gastrointestinal tract. In the United States, the incidence of UC is 8-12 per hundred thousand population per year(1) and the disease is characterized by continuous colonic mucosal inflammation involving the rectum. Patients classically present with abdominal pain and bloody diarrhea. CD has an incidence of 5 per hundred thousand population per year(2) and is marked by focal, transmural inflammation involving both the upper and lower gastrointestinal tracts. This transmural inflammation can result in fibrotic and stenotic strictures or penetrating disease with fistula and abscess formation. Characteristic

Table 1. Association results for Crohn's disease risk loci

SNP	Chromosome	Gene of Interest
rs11209026	**1p31**	***IL23R***
rs2476601	1p13	*PTPN22*
rs4656940	1q23	*CD244, ITLN1*
rs7517810	1q24	*TNFSF18, TNFSF4, FASLG*
rs7554511	1q32	*C1orf106, KIF21B*
rs3792109	**2q37**	***ATG16L1***
rs3197999	3p21	*MST1, GPX1, BSN*
rs11742570	5p13	*PTGER4*
rs12521868	5q31	*SLC22A4, SLC22A5, IRF1, IL3*
rs7714584	**5q33**	***IRGM***
rs6556412	**5q33**	***IL12B***
rs6908425	6p22	*CDKAL1*
rs1799964	**6p21**	***LTA, HLA-DQA2, TNF, LST1, LTB***
rs6568421	**6q21**	***PRDM1***
rs415890	6q27	*CCR6*
rs1456896	7p12	*IKZF1, ZPBP, FIGNL1*
rs4871611	8q24	
rs10758669	**9p24**	***JAK2***
rs3810936	**9q32**	***TNFSF15, TNFSF8***
rs12242110	10p11	*CREM*
rs10761659	**10q21**	***ZNF365***
rs4409764	**10q24**	***NKX2-3***
rs7927997	11q13	*C11orf30*
rs11564258	12q12	*MUC19, LRRK2*
rs3764147	13q14	*C13orf31*
rs2076756	**16q12**	***NOD2***
rs2872507	**17q21**	***GSMDL, ZPBP2, ORMDL3, IKZF3***
rs11871801	**17q21**	***MLX, STAT3***
rs1893217	18p11	*PTPN2*
rs740495	19p13	*GPX4, SBN02*
rs1736020	21q21	
rs2838519	21q22	*ICOSLG*
rs2797685	1p36	*VAMP3*
rs3180018	1q22	*SCAMP3, MUC1*
rs1998598	1q31	*DENND1B*
rs3024505	**1q32**	***IL10, IL19***
rs13428812	2p23	*DNMT3A*
rs780093	2p23	*GCKR*
rs10495903	2p21	*THADA*
rs10181042	**2p16**	***C2orf74, REL***
rs2058660	2q12	*IL18RAP, IL12RL2, IL18R1, IL1RL1*
rs6738825	2q33	*PLCL1*
rs7423615	2q37	*SP140*
rs13073817	3p24	
rs7702331	5q13	
rs2549794	**5q15**	***ERAP2, LRAP***
rs11167764	**5q31**	***NDFIP1***
rs359457	5q35	*CPEB4*
rs17309827	6p25	
rs1847472	6q15	*BACH2*
rs212388	6q25	*TAGAP*
rs6651252	8q24	
rs4077515	**9q34**	***CARD9, SNAPC4***
rs12722489	10p15	*IL2RA*
rs1819658	10q21	*UBE2D1*
rs1250550	10q22	*ZMIZ1*
rs102275	11q12	*FADS1*
rs694739	11q13	*PRDX5, ESRRA*
rs2062305	13q14	*TNFSF11*
rs4902642	14q24	*ZFP36L1*
rs8005161	14q35	*GALC, GPR65*
rs17293632	**15q22**	***SMAD3***
rs151181	16p11	*IL27, SH2B1, EIF3C, LAT, CD19*
rs3091315	17q12	*CCL2, CCL7*
rs12720356	19p13	*TYK2, ICAM1, ICAM3*
rs736289	19q13	
rs281379	**19q13**	***FUT2, RASIP1***
rs4809330	20q13	*RTEL1, TNFRS-F6B, SLC2A4RG*
rs181359	22q11	*YDJC*
rs713875	22q12	*MTMR3*
rs2413583	22q13	*MAP3K71P1*

The table reports data from the two Crohn's disease GWAS (10, 20)

Table 2. Association results for ulcerative colitis risk loci

SNP	Chromosome	Gene of Interest
rs3806308	**1**	***RNF186, OTUD3, PLA2G2E***
rs1317209	**1**	***RNF186, OTUD3, PLA2G2E***
rs6426833	**1**	***RNF186, OTUD3, PLA2G2E***
rs2201841	**1**	***IL23R***
rs11209026	**1**	***IL23R***
rs10800309	**1**	***FCGR2A, FCGR2C***
rs3024505	**1**	***IL10, IL19***
rs6706689	**2**	***REL, CCDC139, PUS10***
rs13003464	**2**	***REL, CCDC139, PUS10***
rs3197999	3	*MST1*
rs4957048	5	*CEP72, TPPP*
rs2395185	6	*C6orf10, BTNL2*
rs4598195	7	*DLD, LAMB1*
rs4077515	**9**	***CARD9***
rs11190140	**10**	***NKX2-3***
rs1558744	**12**	***IFNG, IL26***
rs971545	**12**	***IFNG, IL26***
rs2305480	**17**	***ORMDL3* region**
rs2836878	21	Near *PSMG1*
rs2476601	1p13	*PTPN22*
rs2274910	1q23	*ITLN1*
rs9286879	1q24	
rs11584383	1q32	*KIF21B*
rs780094	2p23	*GCKR*
rs917997	2q11	*IL18RAP*
rs4613763	5p13	*PTGER4*
rs2188962	**5q31**	***IBD5* locus**
rs13361189	**5q33**	***IRGM***
rs10045431	**5q33**	***IL12B***
rs3763313	**6p21**	***BTNL2, SLC26A3, HLA-DRB1, HLA-DQA1***
rs6908425	6p22	*CDKAL1*
rs12529198	6p25	*LYRM4*
rs17309827	6p25	*SLC22A23*
rs7758080	6q25	
rs2301436	6q27	*CCR6*
rs1456893	7p12	*IKZF1*
rs1551398	8q24	
rs10758669	**9p24**	***JAK2***
rs4263839	**9q32**	***TNFSF15***
rs17582416	10p11	*CUL2, CREM*
rs10995271	**10q21**	***ZNF365***
rs7927894	11q13	*C11orf30*
rs11175593	12q12	*LRRK2, MUC19*
rs3764147	13q14	
rs991804	17q12	*CCL2, CCL7*
rs744166	**17q21**	***STAT3***
rs2542151	18p11	*PTPN2*
rs8098673	18q11	
rs4807569	19p13	
rs1736135	21q21	
rs762421	21q22	*ICOSLG*

The table reports data from the ulcerative colitis GWAS (28)

presentations include chronic bloody or non-bloody diarrhea, abdominal pain, anemia and weight loss.

There is longstanding evidence demonstrating that UC and CD result from an excessive and poorly controlled mucosal immune response to commensal flora in genetically susceptible hosts**.** The inherited susceptibility to developing IBD is complex and thought to be polygenic. Previous studies in monozygotic twins show a higher concordance with CD compared to UC indicating a greater genetic contribution in the risk of developing CD(3). Over the last 5 years, GWAS have revealed numerous IBD susceptibility loci, some of which overlap in CD and UC (Tables 1 and 2). GWAS involve genotyping numerous single nucleotide polymorphisms (SNPs) in patients with UC or CD and comparing them with controls to identify regions of susceptibility through linkage disequilibrium. These regions are then more closely examined to further elucidate which genes are likely to contribute to disease susceptibility. The discovery of susceptibility genes has led to a greater understanding of how dysregulation in innate and adaptive immunity have contributed to the pathophysiology of IBD.

Advances in the understanding of the pathophysiology of IBD reveal that defects in multiple areas including mucus production, epithelial cell barriers, and regulators of the innate and adaptive immune responses serve as susceptibility factors for the development of IBD (Figures 1-3, Table 3). Although CD and UC differ in location and pathology, accumulating evidence suggest that these are not distinct immunologic diseases but that specific

Table 3. Summary of factors and cells that contribute to IBD pathogenesis

	Cells/Factors	Genes Involved	Physiologic Function	Pathogenesis	Activation	Suppression	Produces
Microbiome	*Bacteroides fragilis*[43]	-	Induction of Foxp3 Treg dependent IL-10 production	-	-	-	-
	Segmented filamentous bacterium[45]	-	Induction of Th17 lymphocytes	-	-	-	-
	Norovirus	*ATG16L1*[163], *FUT2*[36-38]	Potential environmental trigger	Infection in mice with hypomorphic ATG16L1 expression results in abnormal Paneth cell function	-	-	-
Epithelial Cell Barrier and Microbial Host Interface	Epithelial Cell	*OCTN1*[50], *OCTN2*[50], *DLG5*[52], *MAGI2*[53-55]	Forms protective barrier	Alterations in genes can affect epithelial cell integrity	-	-	
		NOD2/CARD15[65-70]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	MDP	-	
		TLR8[57-61]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	Single stranded RNA		
		TLR9[62-64]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	CpG islands		
	Paneth Cell	*NOD2/CARD15*[70-72] *ATG16L1*[70-72,75,82]	Secretion of antimicrobial peptides	Reduced production of defensins	-	-	-
		TCF-4[73-74]	Transcription factor	Lower levels associated with lower defensin levels Variants in the promoter associated with small bowel CD	-	-	-
		TNFSF15[85-86]		Overexpression increases Paneth cell numbers			
		IRGM[75-80]	Involved in autophagy	Diminished autophagic dependent granule formation			
	Goblet Cell	*MUC2*[48-49]	Secretion of mucin	Alteration in mucin production can compromise barrier function	-	-	-
		TNFSF15[86]		Overexpression increases goblet cell expression			
	Alpha (1,2) Fucosyltransferase	*FUT2*[22]	Catalyzes fucose molecule to oligosaccharides Regulates secretion of H1 antigens	May alter how commensal or pathogenic organisms adhere to the epithelium	-	-	-
Innate Immunity	Dendritic Cells	*NOD2/CARD15*[65-70]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	MDP	-	IL-23 IL-12
		ATG16L1[75-76,81-82]	Involved in autophagy	Diminished autophagy	-	-	
		IRGM[75-80]	Involved in autophagy	Diminished ability to clear intracellular pathogens			
		TLR8[57-61]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	Single stranded RNA		
		TLR9[62-64]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	CpG islands		
	Monocytes and Macrophages	*NOD2/CARD15*[65-70]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	MDP	-	IL-23 IL-12
		ATG16L1[75-76,81-82]	Involved in autophagy	Diminished autophagy	-	-	
		IRGM[75-80]	Involved in autophagy	Diminished ability to clear intracellular pathogens			
		TLR8[57-61]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	Single stranded RNA		

		TLR9[62-64]	Nonspecific pattern recognition receptor	Diminished ability to detect intracellular bacteria	CpG islands		
Innate-Adaptive Interface	TL1A	*TNFSF15*[84-86,91,103,106,107-109]	Enhances Th1 and Th17 effector function Expands Treg cells but diminishes Treg function	Overexpression leads to uncontrolled mucosal inflammation	Fc gamma receptors, TLRs, NLRs	TLR 8 signaling	
Adaptive Immunity	Th17 lymphocytes	*IL-23R, JAK2, STAT3, IL-12B, TNFSF15*[10,20]	Production of pro-inflammatory cytokines	Persistent activation leads to robust production of inflammatory cytokines leading to mucosal inflammation	IL-23 IL-6 TGF-B		IL-17A, IL-17F, IL-21. IL-22
	Th1 lymphocytes	*IL-12B, IFN-gamma*[10,20,28]	Production of pro-inflammatory cytokines	Persistent activation leads to robust production of inflammatory cytokines leading to mucosal inflammation	IL-12		IFN-gamma TNF-alpha
	Th2 lymphocytes	*?*	Production of pro-inflammatory cytokines	Persistent activation leads to robust production of inflammatory cytokines leading to mucosal inflammation	IL-4		IL-4, IL-5, IL-13, IL-25

alterations in overlapping pathways drive the disease towards the phenotype characteristic of UC or CD. The susceptibility genes highlighted from GWAS have guided current translational research and consequently allowed for a better understanding of IBD pathophysiology.

3. GENETICS

The field of IBD genetics is the fastest moving of all IBD research. Until recently geneticists struggled to advance the field beyond epidemiological studies pointing to a genetic contribution in IBD. Previously, only broad regions of the human genome likely to contain susceptibility genes were identified with the exception of *NOD2/CARD15*, the first susceptibility gene found in IBD. Two groups of investigators published successive papers identifying the association between CD and NOD2(4, 5). It took 5 years for a European group that fine-mapped a linkage region on chromosome 16 to identify *NOD2* polymorphisms as the causative SNPs (4). Using linkage peaks, the North American genetics consortium recognized that *CARD15* would be a good susceptibility candidate gene because of its involvement in intracellular pathogen recognition(5). Four years later researchers saw the next major advance and the dawn of a new era of rapid gene identification.

A number of advances beyond the field of IBD genetics facilitated the new era of GWAS including the Human Genome Project, the HapMap Project and the development of chip-based genotyping technology. These three factors not only resulted in researchers having a reference road map of the human genome, but also a significantly more efficient genotyping platform. This platform allows large-scale studies examining the whole genome in a hypothesis free manner to be performed. The first study to take advantage of these advances was performed by modern standards, in a modestly sized Japanese CD population in 2005. This study identified a strong association between CD and genetic variation in the *tumor necrosis factor super family 15* (*TNFSF15*) gene, a finding reproduced in 2 cohorts of British Caucasian CD patients as well as in a British UC population(6). The *TNFSF15* association with IBD has subsequently been reproduced in a number of Caucasian-based studies(7-10) and is clearly the dominant gene in the East-Asian population(11, 12). The *TNFSF15* locus is also associated with susceptibility to leprosy in the Asian population suggesting a link between infectious conditions and IBD(13). The exact *TNFSF15* variants that predispose to IBD remain to be identified and there are no obvious non-synonymous (amino acid changing) SNPs in this region identified to date.

IBD has been at the forefront in the utilization of GWAS. The first GWAS in the Caucasian population was performed by the North American IBD genetics consortium in which variants in the *IL-23 receptor* (*IL-23R*)(14) and the autophagy related molecule *immunity-related GTPase family M* (*IRGM*)(15) genes were identified. Autophagy was further implicated by another study in the Caucasian population that identified a non-synonymous SNP in *ATG16L1* as a susceptibility variant(16). Additional studies from the U.K.(17, 18) and a French-Belgian CD GWAS(19) identified further variants associated with CD susceptibility including additional variants within the IL-23/Th17 pathway such as *IL-12B* and other genes related to novel pathogenic pathways such as *PTGER4*.

In an effort to increase the power of the studies, the 3 Caucasian GWAS already mentioned were combined in a meta-analysis of over 3000 cases and approximately 5000 controls with an adequately powered independent replication cohort. This study resulted in the identification of over 30 loci (including the ones identified in earlier GWAS) although this explained only 20% of the total genetic contribution in this population(20). This study further emphasized the role of the IL-23/Th17 pathway in CD pathogenesis through the identification of *STAT3*, and *JAK2* as susceptibility genes. The penchant for CD GWAS showed little sign of abatement with further studies performed in both the pediatric and adult populations. A

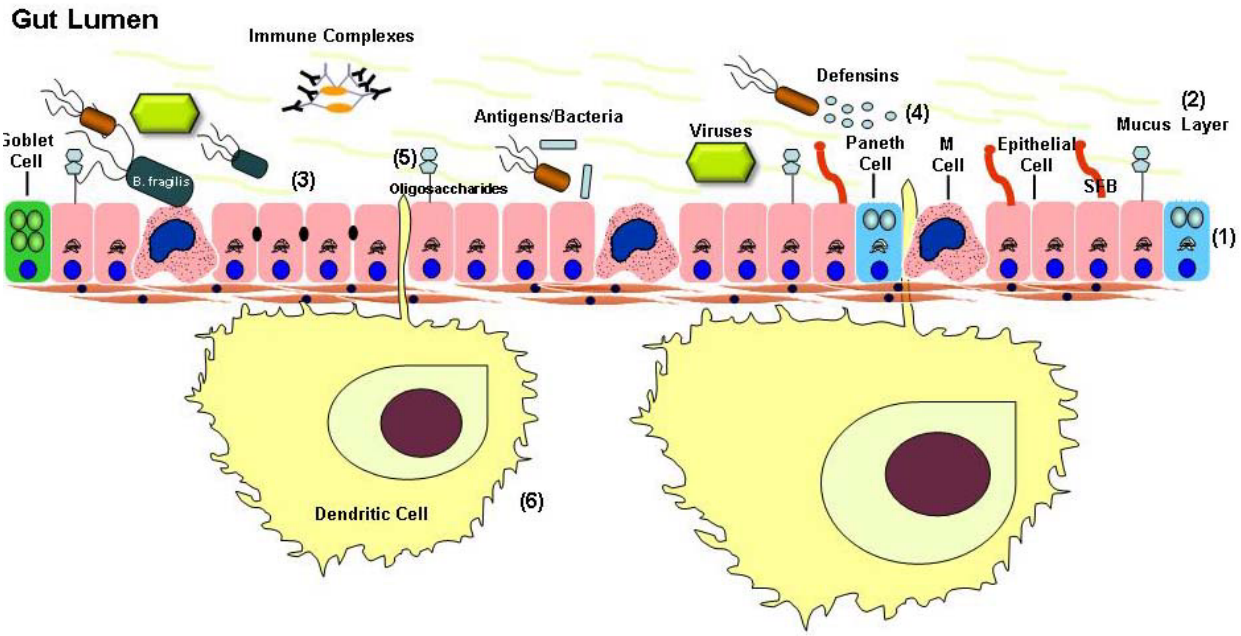

Figure 1. The intestinal immune system is protected from antigenic stimulation by the intestinal epithelial cell layer (1) and mucus layer (2). Defects in epithelial cell integrity may result from alterations in genes encoding OCTN1, OCTN2, DLG5, and MAGI2 (3) which may result in free passage of microbes across the epithelial layer from the intestinal lumen. Paneth cells located at the base of the small intestinal crypts help minimize pathogenic invasion through secretion of defensins. Genes that relate to Paneth cell function include *NOD2/CARD15, ATG16L1, IRGM, TNFSF15* and *TCF-4*. Microbial adherence to the epithelial layer may occur through host surface oligosaccharides (5). Alterations in *FUT2* may affect the way that commensal and pathogenic organisms including norovirus adhere to the epithelium. Passage of pathogens across the epithelial layer results in activation of innate immune cells such as dendritic cells (6) that can then activate the adaptive immune response and production of inflammatory cytokines.

pediatric study identified *IL-27*(21) as an additional locus and was able to show association between this pediatric population and over 20 of the 32 loci identified in the adult population. This suggests that similar genes are associated with early and late onset disease. A further study demonstrated an association between Fucosyltransferase 2 (FUT2) non-secretors and CD(22) further implicating the importance of the mucus layer in host-microbial interactions.

The IBD international genetics consortium combined CD GWAS containing over 6000 CD cases and approximately 50,000 total individuals (cases and controls in both index and replication cohorts) resulting in the identification of a total of 71 confirmed loci for IBD(10). A number of interesting genes were implicated in this study (Table 1) including genes further emphasizing the importance of the IL-23R/Th17 pathway (*TYK2*) as well as highlighting other processes including the TGF-beta pathway (*SMAD3*), endoplasmic reticulum stress (*NDIF1P*), T cell activation (*TAGAP*) and macrophage activation (*VAMP3*) among others. A number of other findings from this study included the calculation that these 71 loci only accounted for approximately 25% of the genetic variation in CD. This is likely to be a significant underestimate of the true contribution as the associations identified in this study are likely to have only partially tagged the true causative SNPs. The best example of this is the *NOD2/CARD15* locus, tagged by a single SNP, but is known to have three common SNPs within the locus associated with CD(23). The tagged SNP from the meta-analysis contributes 0.8% of the heritability of CD in this study whereas earlier studies examining this locus in more detail suggest that *NOD2/CARD15* contributes approximately 5% of the heritability. Many of the implicated loci were already known loci for a number of infective (e.g. tuberculosis, leprosy, norovirus, *Helicobacter pylori*, and hepatitis C) and immune related conditions (e.g. celiac disease, ankylosing spondylitis, psoriasis, and UC).

Similar progress in UC disease identification has been slower in part because of the less heritable nature of the disease. Nevertheless, GWAS identified of a number of genes including *interferon gamma* (*IFN-gamma*), *IL-10*, *IL-23R*, *FcGR2a* and the known HLA region that lead to UC susceptibility(24-27). More recently a meta-analysis of 3 GWAS has implicated some novel genes associated with UC including *CARD9* and *orosomucoid1-like 3* (*ORMDL3*)(28). In this study, association was seen with the 14 previously identified UC loci. More than half the known CD loci from the initial CD meta-analysis showed association with UC bringing the total known number of UC loci to over 30 (Table 2). Studies of a similar size to the latest CD meta-analysis(10) are in progress in UC with the promise of additional gene discovery.

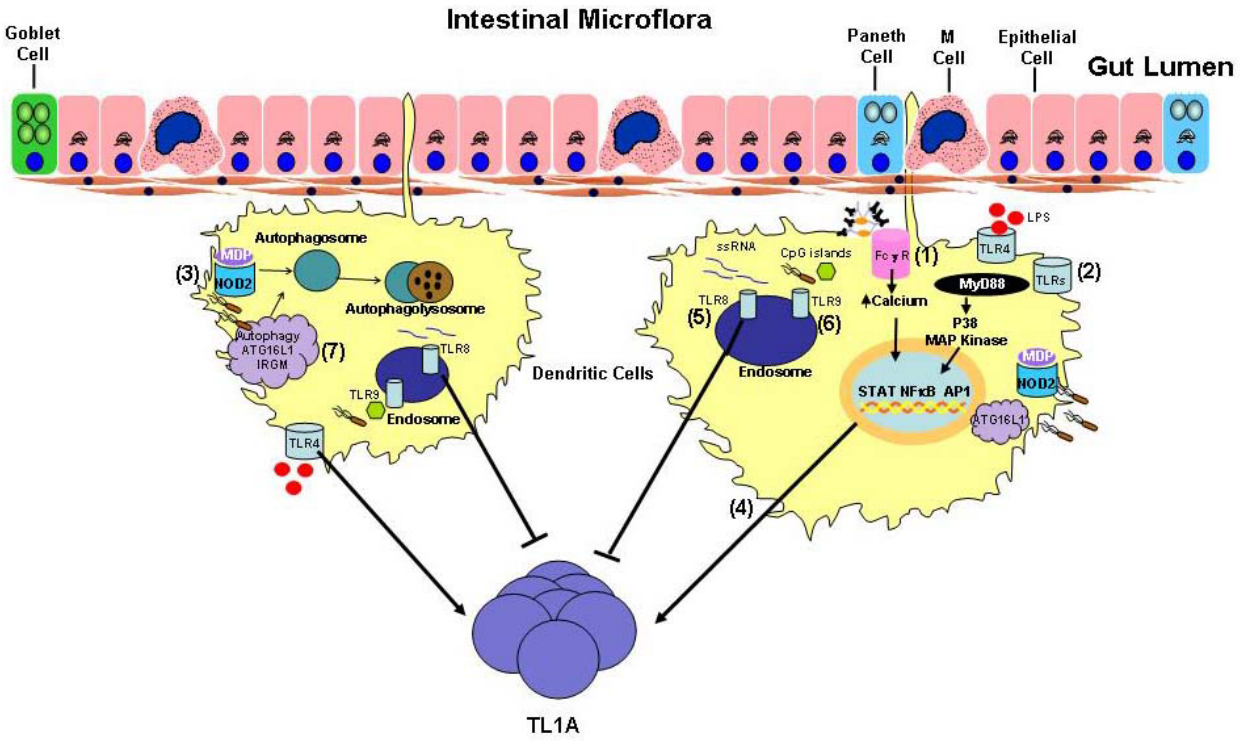

Figure 2. Activation of the innate immune response occurs when organisms breach the epithelial cell barrier. Microbial activation of innate immune cells such as dendritic cells can occur through different signaling pathways including Fc gamma receptors (1), Toll like receptors (TLRs) (2) and NOD2 (3). Signaling through Fc gamma receptors and certain TLRs results in induction of TL1A expression (4). Activation of TLR8 negatively regulates TL1A expression (5) which may make it a useful therapeutic target in the future. Impaired microbial clearance may result from alterations in pattern recognition receptors (2,3,5,6) or autophagy (7) leading to persistent activation of innate immune cells.

The identification of loci through genetic studies is only the first step in a multi-faceted pathway to understanding how variation in these genes lead to an increased risk of developing chronic mucosal inflammation. Fine-mapping genetic studies in large cohorts will be needed to elucidate the precise disease associated variants and these projects may require large-scale sequencing efforts. Once the causative polymorphisms are identified, concerted efforts to understand the functional consequences of the genetic variation will be necessary. It is important to recognize that studies looking for variation in nucleotide sequences are but one aspect of genetic contribution to disease. Other levels of genetic variation that will affect disease susceptibility include epigenetic variation which only little work has been performed in IBD(29). Furthermore, given that IBD is a complex trait with both genetic and environmental aspects, researchers will need to assess host genetic risk and environmental interactions such as smoking and the microbiome and incorporate them into their analyses. Although there is a still a significant amount of work to be done, clinicians and patients will see some clinical benefits in return for the investment in genetic research in IBD.

4. MICROBIOME

4.1. Characterization of the microbiota

Of the 10-100 trillion organisms that reside within a human colon, the vast majority are bacteria but they also include viruses and members of Archaea. The relationship between the intestine and commensal organisms is one of symbiosis. The intestine provides nutrition to the bacteria, which in return aid in digestive processes by breaking down non-absorbable complex sugars and xenobiotics and producing a variety of compounds such as short chain fatty acids and vitamins. In addition, these organisms help develop and modulate the immune response and minimize pathogenic invasion(30).

Advances in 16S ribosomal RNA (rRNA) gene and metagenomic sequencing provide extensive information regarding the prevalent species and their microbial gene functions that make up the intestinal microbiome. Metagenomic sequencing allows for the cataloging of microbial sequences from fecal samples, which provides insight into the species types and prevalent microbial genes that make up the intestinal flora. Bacteroidetes and Firmicutes comprise 90% of the gut microbiota(31). This was confirmed by another study in which DNA from fecal specimens from a 124 healthy, overweight/obese and individuals with IBD were analyzed. Prominent clusters at the genus and family levels included bacteria of the Bacteroidetes and *Dorea/Eubacterium/Ruminococcus* groups, bifidobacteria, proteobacteria and streptococci/lactobacilli(32). Individuals with IBD have a decrease in bacterial diversity

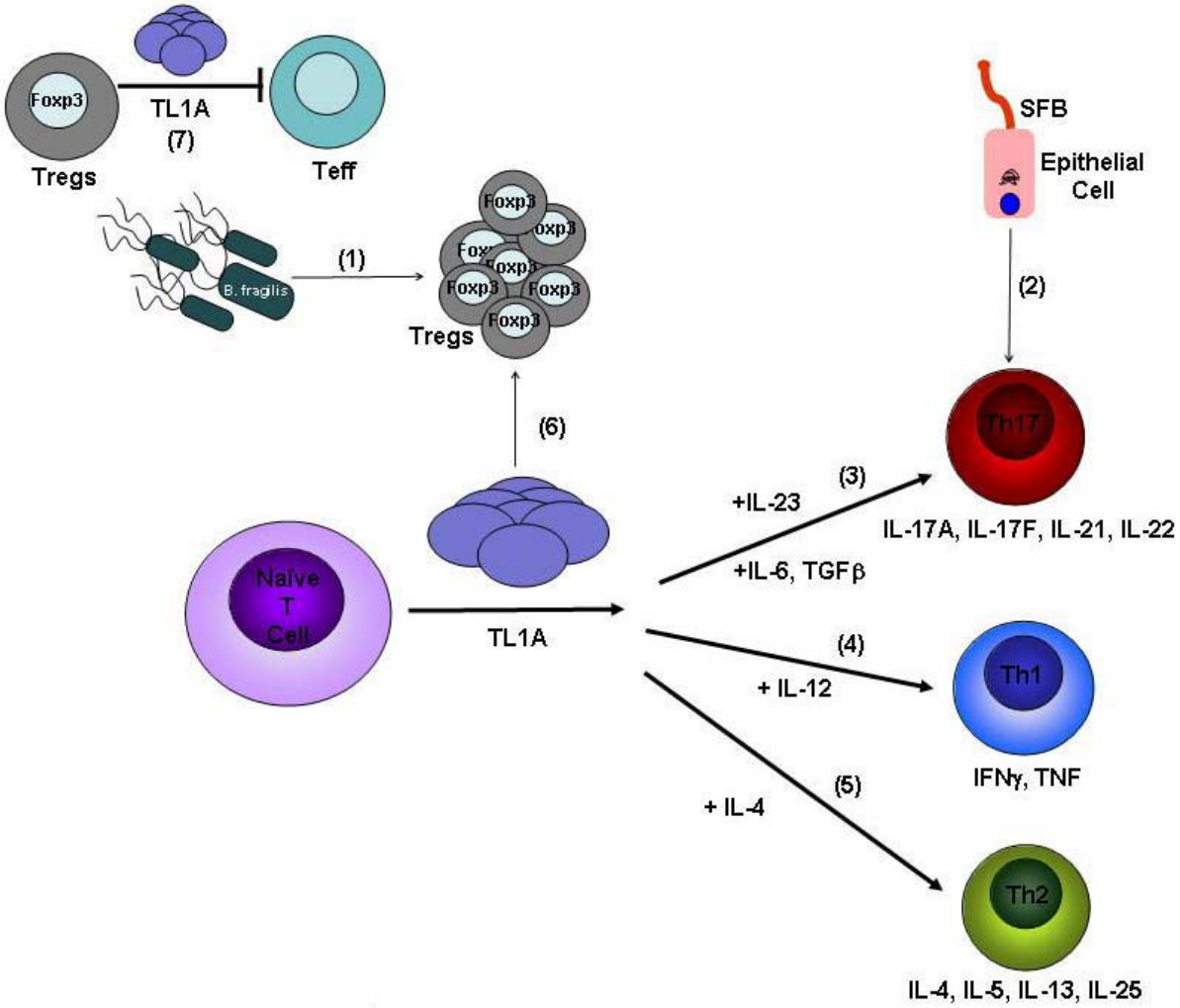

Figure 3. Modulation of the adaptive immune response occurs by the intestinal microflora such as *B. fragilis* (1) and SFB (2) through induction of T regulatory (Treg) and Th17 lymphocytes respectively. TL1A in combination with IL-23, IL-6 and TGF-beta induces generation of Th17 lymphocytes (3) while in combination with IL-12 and IL-4 enhances the development of Th1(4) and Th2 (4) lymphocytes respectively. Th1, Th2 and Th17 lymphocytes produce specific effector cytokines (indicated in the figure) that characterize the cytokine milieu seen in CD or UC. TL1A also appears to increase the number of Treg lymphocytes (6) but diminishes their suppressive ability on T effector (Teff) cells (7). The ability of TL1A to expand Teff lymphocytes and suppress Treg function may make it an ideal therapeutic target in treatment of IBD.

evidenced by a fewer number of non-redundant bacterial genes compared to healthy controls. In addition, the bacterial profile not only differs between individuals with IBD and healthy controls but also between individuals with UC versus CD. There are a decreased number of commensal organisms including the Lachnospiraceae and Bacteroidetes and an increase in the number of Proteobacteria in individuals with IBD(33).

Microbial genes utilized for bacterial function are also catalogued and separated into the minimal gut genome and the minimal gut metagenome. The minimal gut genome is the set of microbial genes required for bacterial function in the gut and the minimal gut metagenome are genes involved in the homeostasis of the entire ecosystem across species. The bacterial genes required for gut function include genes for adhesion to the host such as collagen, fibrinogen and fibronectin and genes encoding proteins needed for harvesting sugars from the host(32). Other metagenomic genes include phage-related proteins, which suggest a required role for bacteriophages in the intestinal environment and genes related to the biosynthesis of amino acids and vitamins provided by commensal bacteria.

4.2. Host-microbial interactions

Host-microbe interactions are crucial in the development and modulation of the immune system and protection from pathogenic bacterial invasion. The host has evolved a variety of mechanisms to minimize the exposure of the intestinal epithelial surface to pathogenic organisms. Such factors include the compartmentalized

mucus layer, the intestinal epithelial cell (IEC) layer and the secretion of a variety of proteins and antimicrobial peptides(34).

Both commensal and pathogenic bacteria have evolved a number of mechanisms to aid in adherence to the epithelial cell layer, including the use of host cell surface molecules such as oligosaccharides. The ability of commensal organisms to adhere to the epithelial layer helps deter invasion by displacing pathogenic bacteria. *FUT2* is a gene located on chromosome 19 that encodes a type alpha (1,2) fucosyltransferase. This enzyme regulates the secretion of the H1 antigen of the ABO antigens into the gastrointestinal mucosa by catalyzing the addition of a fucose molecule to oligosaccharides. Twenty percent of individuals are non-secretors and are unable to secrete the H1 antigen into bodily fluids such as saliva(35). The FUT2 non-secretor status is associated with a variety of illnesses, including recurrent infections with norovirus (36-38) and encapsulated organisms(39, 40), duodenal ulcerations(41) and CD susceptibility (22). The inability to secrete these ABO antigens into the gastrointestinal mucosa can affect how both commensal and pathogenic flora interact with the epithelial cell layer which may alter the mechanism by which the host minimizes pathogenic invasion. Specifically in IBD, the inability of commensal organisms to adhere to the epithelial layer and displace pathogens may result in increased susceptibility to infection, invasion and activation of the innate and adaptive immune response.

Commensal bacteria are involved in the development and modulation of the host immune system by promoting the development of certain lymphocyte subsets and suppression of others(34). Sampling of the microbial antigens in the intestine occurs by a variety of mechanisms including via Fc receptors, microfold (M) cells and antigen presenting cells (APC) such as dendritic cells (DC). This sampling allows the microbiota to modulate the intestinal immune response. DC sample intraluminal antigens by extending processes through the intestinal epithelial layer and present their displayed antigen to corresponding T lymphocytes.

Bacteroides fragilis (*B. fragilis*) normally resides within the large intestine and can act either as a commensal or pathogenic organism depending on the production of a metalloprotease toxin(42-44). The capsule polysaccharide A (PSA) of the commensal form of *B. fragilis* is thought to modulate the immune response to prevent the development of autoimmune-mediated diseases such as CD and experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis(43, 44). PSA induces regulatory T lymphocytes (Tregs) that secrete IL-10, a negative regulator of mucosal inflammation(43).

The effect of *B. fragilis* on the induction of Foxp3+ Tregs and IL-10 production was studied using irradiated C57BL/6 mice reconstituted with bone marrow from Foxp3-GFP mice and exposed to three conditions: (1) germ-free (2) monocolonization with wild-type *B. fragilis* or (3) monocolonization with a strain of *B. fragilis* without PSA(43). In germ-free conditions, there is minimal mRNA production of IL-10. Colonization with *B. fragilis* yields the highest degree of PSA-dependent IL-10 production and results in a 2-fold PSA-dependent increase in the number of Foxp3+ Tregs. PSA promotes differentiation of CD4+CD25+Foxp3- T cells into CD4+CD25+Foxp3+ Tregs. Furthermore, mice with TNBS-induced colitis treated with PSA exhibit a correction in weight loss and an improvement in colonic inflammation. This improvement is marked by increased IL-10 and Foxp3 and decreased IL-17A mRNA expression in the gut. The ability of PSA to induce production of IL-10, an anti-inflammatory cytokine in addition to ameliorating TNBS induced colitis may make it a potential therapeutic agent in the treatment of patients with IBD.

Segmented filamentous bacterium (SFB) is commensal, spore-forming, gram-positive organism of the genus *Arthromitus* that is found in the gut and is most closely related to bacteria from the genus *Clostridia*(45). Observations that C57BL/6 (B6) mice from different commercial vendors exhibit different proportions of Th17 cells in the small intestinal lamina propria (LP) led to the discovery that mice expressing a higher number of Th17 cells are colonized with SFB(45). Furthermore, mice lacking Th17 cells in the small intestinal LP under germ-free conditions develop increased numbers of Th17 cells when colonized with feces from mice monocolonized with SFB and not other bacteria. SFB induces production of IL-22 and IL-17A but not IFN-gamma in CD4+ T cells. Colonization with SFB induces mRNA transcripts of serum amyloid A, an acute phase reactant. Recombinant forms of amyloid A added to co-cultures of naïve CD4+ T cells and LP DC induces Th17 differentiation evidenced by induction of IL-17A, IL-17F, IL-21, IL-22 and the transcription factor retinoic acid–related orphan receptor (ROR) gammaT. The presence of SFB also reduces the capacity of orally administered *Citrobacter rodentium* to grow and invade colonic epithelial cells(45). The ability of this commensal microbe to induce a Th17 response and to help protect against pathogenic invasion is further evidence that supports the role of the microbiota in modulation of the immune response. However, exuberant Th17 activation by such a commensal microbe can lead to the robust inflammatory response characteristic of CD. Thus, it is possible that manipulation of the number of SFB that colonize the gastrointestinal tract may alter the course of Th17 associated inflammatory diseases such as CD.

The intestinal microbiota is implicated in priming and enhancing the effects of the innate immune response. Pattern recognition receptors like Toll-like receptors (TLRs) and cytosolic Nod-like receptors (NLRs) play an important role in the detection and handling of bacteria. Commensal microflora are a source of peptidoglycan (PGN) that can act to enhance killing of *Streptococcus pneumoniae* (*S. pneumoniae*) in a NOD1-dependent manner by bone marrow-derived neutrophils(46). To determine the effect of meso-diaminopimelic acid (meso-DAP) PGN on neutrophil activation, neutrophils isolated from mice exposed to attenuated *Haemophilus influenzae* (*H. influenzae*) were used in an *ex vivo* opsonophagocytic assay with *Staphylococcus aureus* (*S. aureus*). These

neutrophils exhibit a NOD1-dependent enhanced ability to kill *S. aureus* compared to unexposed controls. Unstimulated neutrophils isolated from NOD1 knockout mice have diminished baseline ability to kill opsonized bacteria. The source of meso-DAP PGN that modulates neutrophil activation is from normal intestinal microflora as neutrophils isolated from bone marrow of mice treated with broad-spectrum antibiotics exhibit diminished ability to kill *S. pneumoniae* and *S. aureus*. The effects of meso-DAP PGN on neutrophil activation is systemic as mice colonized with tritium-labeled PGN from *Escherichia coli* (*E. coli*) exhibit accumulation of labeled PGN in the sera, bone marrow and in neutrophils 72 hours after inoculation(46). These findings support the concept that the intestinal microbiome can modulate and prime the innate and adaptive immune system. The priming of the host's immune response can allow for rapid activation and enhanced killing when exposed to pathogenic organisms.

5. ALTERATIONS IN BARRIER FUNCTION

The innate immune response is the host's first line of nonspecific defense and does not result in the development of long-term immunity. The gastrointestinal innate immune system is comprised of a mechanical barrier consisting of the mucus layer and epithelial cell barrier, phagocytes, natural killer cells, proteins and receptors involved in recognition of basic bacterial and viral motifs and autophagic mechanisms. Likewise, proteins such as defensins are secreted across this barrier by IECs and Paneth cells which aid in killing bacteria and other pathogenic organisms.

5.1. Mucus layer

Goblet cells secrete mucin, the principal component of the mucus layer, which further comprises two sub-layers – an inner layer adjacent to the IECs that is generally free of bacteria and an outer layer that is colonized with bacteria. The mucus layer forms a barrier that protects the epithelial cells from exposure to pathogens. To date, there have been 21 different mucin genes identified(47). MUC2 constitutes the majority of the mucin produced. In patients with IBD, both the production and composition of mucin are altered. Data suggest that in patients with UC the mucus layer may be thinner, and the amount of secreted MUC2 is reduced which may be related to the loss of goblet cells(48). MUC2 knockout mice treated with dextran sodium sulfate (DSS) develop a more severe colitis compared to wild-type controls(49). Alterations in the composition or thickness of the mucus layer could contribute to the pathogenesis of IBD by facilitating exposure of the epithelial cell layer to pathogenic bacteria.

5.2. Alterations in the epithelial cell barrier

The IEC barrier consists of epithelial cells linked by tight junctions and forms a semi-permeable barrier, which allows nutrients to cross but prevents penetration by luminal bacteria. CD susceptibility genes, *organic cation transporter (OCTN)* and 2 types of membrane associated guanylate kinases (MAGUK) relate to the integrity of the epithelial cell barrier.

Genes encoding the OCTN 1 and 2 transporters are located with the cytokine gene cluster known as the *IBD5 locus* on chromosome 5. These transporters are involved in transport of cationic proteins including amino acids and nutrients like carnitine. Two variant alleles that result from mutations within the *OCTN1* and *OCTN2* genes create proteins defective in cellular transport and lead to increased susceptibility to CD. The odds ratio for CD susceptibility is greatest in patients exhibiting mutations in genes encoding both *OCTN* and *NOD2*(50). This susceptibility is thought to result from impaired fatty acid oxidation, which in the setting of bacterial antigen exposure may cause colitis in experimental models(30, 50, 51).

MAGUKs are proteins with a guanylate kinase domain that act as a scaffold at sites of cell contact and interact with tight junctions and the cytoskeleton. *Drosophila discs large homologue 5 (DLG5)* is a gene located on chromosome 10 and encodes a MAGUK. DLG5 acts as a scaffold for proteins taking part in signal transduction and helps maintain the structure of the epithelial barrier. There are two haplotypes of the gene, one with an amino acid substitution and is associated with IBD and a second that is under-transmitted in individuals with IBD(52). *Membrane associated guanylate kinase WW and PDZ containing protein 2* (*MAGI2*) is located on chromosome 7 and encodes a scaffold protein involved in epithelial cell integrity(53). It may also play a role in cell migration and proliferation via phophatase and tensin homolog (PTEN)(54). Variants in *MAGI2* are associated with UC and CD(55) and defects in MAGI2 may result in altered epithelial barrier function however, functional studies are needed to confirm this theory. Defects in epithelial cell integrity may contribute to IBD pathogenesis by allowing free passage of organisms across the epithelial layer where they can persistently activate the innate immune response.

6. ALTERATIONS IN INNATE IMMUNITY

6.1. Pattern recognition receptors

The lack of specificity of the innate immune response requires the recognition of conserved molecular patterns of various antigens called pathogen associated molecular patterns (PAMPs). PAMPs are recognized by pattern recognition receptors such as toll-like receptors (TLRs) and cytosolic Nod-like receptors (NLRs).

In humans there are ten known TLRs some of which are membrane-bound while others are located within the intracellular compartment. The ligands for TLRs are specific and are often components of various microbial and viral products. Signaling through a majority of TLRs results in the production of inflammatory cytokines such as TNF-alpha and type I interferons. Production of type I interferons occurs independently of NF-KB and MyD88(56). TLR2 is membrane-bound and is activated by a number of microbial components, one of which is PGN. Activation of TLR2 drives Th1 production of IL-12 and ultimately IFN-gamma and TNF-alpha. TLR3, TLR7, TLR8 and TLR9 are located within endosomes in the intracellular compartment and TLR7, TLR8 and TLR9 can

activate or inhibit the inflammatory cascade(57-59). The endosomal TLRs detect a number of different microbial components including single and double stranded RNA and CpG islands.

The gene encoding TLR8 lies on the X chromosome and is an IBD susceptibility gene. Haplotypes within the *TLR8* gene confer protection (H1) or risk (H4) to the development of IBD(57). TLR8 agonists can activate pro-inflammatory cytokines such as IFN-gamma, IL-12 and TNF-alpha in peripheral blood mononuclear cells (PBMC)(57, 60). TLR8 can also influence T regulatory lymphocytes, which suppress the immune response through production of factors like IL-10. Ligands that activate the TLR8 pathway, such as single stranded RNA, can reverse the normal function of these regulatory lymphocytes(61). TLR8 agonists can also curb the inflammatory cascade through inhibition of TL1A expression induced by Fc gamma receptor signaling. The finding that *TLR8* variants can protect against or predispose to the development of IBD coupled with its dual role in inducing and inhibiting critical inflammatory cytokines implicates its role as a modulator of inflammation in IBD. Future therapeutic agents that target TLR8 signaling may be beneficial in the treatment of IBD.

TLR9 is another endosomal TLR that recognizes CpG islands in bacterial and viral genomes. Like TLR7, TLR9 uses adaptor proteins that allow it to move from the endoplasmic reticulum (ER) to endolysosomes. TLR9 can activate pro-inflammatory cytokines and type I interferons through two different pathways(62). The production of pro-inflammatory cytokines such as IL-12p40 is dependent on NF-KB activation whereas the production of type I interferons is dependent on the adaptor protein 3 complex. Upon ligand stimulation, TLR9 traffics from the ER to an endosome enabling its cleavage and activation of NF-KB, which ultimately results in the transcription of inflammatory cytokines. In order for type I interferon production to occur, the adaptor protein complex 3 interacts with the cleaved TLR9 to allow it to traffic to lysosome related organelles where it induces transcription of interferon genes(62). The gene encoding TLR9 lies on chromosome 3 in the vicinity of a locus that is associated with susceptibility to CD and UC(63). The frequency of the *TLR9* polymorphism -1237C is increased in patients with CD exhibiting mutations in NOD2 and variants in the *IL-23 receptor (IL-23R)*. Epistatic interactions of this polymorphism also occur with *DLG5* variants(64). Polymorphisms in *TLR9* that affect microbial sensing coupled with other alterations in IBD susceptibility genes may impair microbial defense resulting in dysregulation of innate immunity.

The NOD proteins are cytosolic pattern recognition receptors that recognize microbial components. NOD1 is an NLR that is expressed in non-myeloid cells and APC such as DC and macrophages(65). NOD1 is encoded by the *CARD4* gene, and various polymorphisms in *CARD4* are associated with inflammatory disorders such as asthma and IBD. The ligand for NOD1 is meso-DAP PGN, which is a component of gram-negative bacteria. NOD2 is encoded by *CARD15* and detects muramyl dipeptide (MDP), a component of PGN that is present in both gram-negative and gram-positive bacteria. Stimulation of NOD1 and NOD2 results in the production of inflammatory cytokines through activation of the NF-KB or mitogen-activated protein kinase (MAPK) pathways(65).

NOD2/CARD15 is one of the first discovered CD susceptibility genes. Homozygous mutations occur in 15% of patients with CD and contribute to the development of granulomatous small bowel disease(4, 5). There are three main mutations resulting from amino acid substitutions in the receptor that lead to an inability to recognize its ligand(66). NOD2 plays a multifaceted role in innate immunity and it is unclear if mutations in NOD2 result in a loss or gain of function(65).

Given that NOD2, an intracellular receptor, and TLR2, a membrane-bound receptor are both activated by PGN, it is possible that an interaction between the two modulate the immune response. MDP activation of NOD2 negatively regulates TLR2 activation by PGN by diminishing IL-12 production in APC(67) and protects mice from the development of TNBS and DSS colitis(68). Similarly, splenocytes isolated from NOD2-deficient mice exhibit enhanced production of IL-12 and IFN-gamma when stimulated with PGN(67). Thus, in the absence of NOD2 function, activation of TLR2 by PGN results in increased IL-12 production.

A second role for NOD2 relates to its loss of function in IECs and Paneth cells. IECs exhibiting mutations in NOD2 have a decreased ability to control the overgrowth of *Salmonella enterica* (*S. enterica*) in the gastrointestinal tract(69). This discovery is thought to be due to defective secretion of defensins by Paneth cells. Ileal biopsies from inflamed tissue in patients with CD have decreased expression of human alpha defensin 5 and 6 (HD-5 and HD-6) with a greater decline occurring in patients with mutations in NOD2(70). Although this finding has been replicated in other studies, there is some concern that the loss of defensins results from loss of the epithelial layer in inflamed mucosa rather than NOD2 expression(71).

Lastly, mutations in NOD2 can also result in a gain of function. The Leu1007fsinsCys (L1007fs) mutation results in a frameshift after the insertion of a cysteine residue and is the most prevalent mutation in CD. Knockin mice expressing the L1007fs mutation in NOD2 are more susceptible to the development of DSS colitis and stimulation of macrophages isolated from these mice with MDP produce more IL-1beta, a pro-inflammatory cytokine(65).

NOD2, like the other pattern recognition receptors, plays an important role in the detection of intracellular invasion by pathogens. Alterations in these receptors impair the ability to sense organisms and may lead to defective microbial clearance and persistent antigenic stimulation of the immune response resulting in the mucosal inflammation and cytokine milieu typically seen in IBD.

6.2. Paneth cells

Paneth cells are found mainly at the base of the crypts within the small intestine, although Paneth cell metaplasia can occur within the colon in setting of chronic inflammation. These cells are characterized by eosinophilic granules that contain alpha defensins. Defensins are antimicrobial proteins that bind to the cell membrane to create a hole and allow for leakage of nutrients and electrolytes. Paneth cells secrete a variety of alpha defensins in addition to lysozymes and secretory phospholipase A2(72). Reduced expression of HD-5 and HD-6 in patients with CD result from several alterations within Paneth cells including mutations in NOD2, decreased expression of transcription factors within the Wnt signaling pathway, and loss of normal autophagy function(70, 72, 73).

The Wnt signaling pathway is activated when the Wnt family protein binds to cell surface receptors. This receptor activation causes translocation of beta-catenin to the nucleus in order to form a complex with factors such as TCF-4. TCF-4 then acts as a transcription factor to control expression of various genes including those related to cell differentiation and production of defensins(73, 74). Lower levels of TCF-4 expression are associated with lower alpha defensin levels in ileal CD, independent of NOD2 mutations and ileal inflammation(74) and variants in the *TCF-4* promoter are associated with small bowel CD(73). There are several IBD susceptibility genes that relate to Paneth cell function such as *NOD2/CARD15*, *ATG16L1, IRGM, TNFSF15* and *TCF-4*. Genetic alterations that result in defective secretion of defensins impairs microbial defense, which renders the host more susceptible to pathogenic invasion.

6.3. Autophagy

The process of autophagy is not only involved in microbial defense but also in the degradation and elimination of normal intracellular components. Autophagy is dependent on autophagosomes and lysosomes. Autophagosomes are double-membraned vacuoles in which contents that need to be degraded are sequestered. These vacuoles fuse with lysosomes in order for the process of degradation by hydrolases to occur. The formation of the autophagosome relies on the generation of a complex of autophagy related proteins (ATG) including ATG5, ATG12 and ATG16L1 at the cellular membrane(75). During the process of autophagy, the addition of lipids and phosphatidylethanolamine to the carboxy terminal of microtubule-associated protein 1A/1B/light-chain 3 (LC3) allow it to localize to the autophagic membrane. Because the amount of lipidated LC3 correlates with the number of autophagosomes, assays detecting lipidated LC3 are used as a surrogate to detect induction of autophagy(76). Autophagic processes are involved in the innate immune response on multiple levels from interactions with TLRs to regulation of the pro-inflammatory cytokines(75).

Two autophagy genes, *IRGM* and *ATG16L1* are implicated in the pathogenesis of IBD. The *IRGM* gene belongs to a family of interferon-inducible immunity-related GTPases (IRGs). It encodes a protein involved in multiple autophagocytic pathways including intracellular clearance of pathogens that may occur through mitochondrial fission(77). There are multiple IRGMs and they are involved in the elimination of intracellular pathogens such as mycobacteria, *Toxoplasma gondii* and *Chlamydia trachomatis*(78, 79). Polymorphisms resulting in alteration of IRGM expression are associated with CD and UC(75, 80).

The autophagy protein ATG16L1 is involved in a number of autophagic pathways such as formation of the autophagosome and exocytosis of secretory granules in Paneth cells. Polymorphisms in the gene encoding ATG16L1 renders susceptibility to the development of CD. The mutation associated with the risk allele results from a threonine to alanine substitution (T300A) in a carboxy terminal domain(75). In mice, deletion of the coiled-coiled domain results in a dysfunctional protein and is lethal as the mice die within the first day of life (81). However, mice hypomorphic for ATG16L1 have lower protein expression and are viable. These mice exhibit Paneth cell abnormalities similar to that found in patients with ileal CD(82). Decreased expression of ATG16L1 results in the induction of cytokines such as type I interferons, IL-1 beta, and IL-18 and defects in the formation and secretion of granules in Paneth cells(75, 82).

The ability to control and eliminate intracellular pathogens is a critical component of the innate immune response and autophagy clearly plays an important role in the clearance of these microbes. Alterations in the autophagic machinery such as in ATG16L1 and IRGM may result in the ineffective elimination of pathogens and the persistent activation of the inflammatory cascade, which may contribute to the pathogenesis of IBD.

7. INNATE AND ADAPTIVE INTERFACE

TNFSF15 encodes the protein TL1A, a member of the TNF superfamily, which binds to death domain receptor 3 (DR3) to initiate a number of immune responses, such as activation and proliferation of T cells resulting in the secretion of pro-inflammatory mediators. TL1A is implicated in the pathogenesis of many autoimmune inflammatory diseases such as asthma and rheumatoid arthritis(83) and accumulating evidence suggest that TL1A plays a critical role in the pathogenesis of IBD. *TNFSF15* contributes susceptibility to IBD in all ethnic groups(83) and may be involved in a differential pattern of inflammation such as that seen in isolated small bowel CD(84-86). Studies by numerous groups reveal that TL1A is a major regulator of mucosal inflammation at the interface between innate and adaptive immune cells and thus is the focus of this section.

Numerous GWAS show *TNFSF15* is a CD susceptibility gene in the Japanese, Korean, European and Jewish populations(6, 8, 12). Haplotypes within the gene confer either risk (haplotype A) or protection (haplotype B) to the development of CD in non-Jewish patients(6, 7, 11, 20). However, in Jewish CD patients who are haplotype B

carriers, there appears to be a trend towards a risk phenotype since these patients exhibit increased disease severity manifested by a higher incidence of surgery and higher antibody responses to the outer membrane porin C of *Escherichia coli* (OMPC)(7, 87, 88). Monocytes isolated from haplotype B carriers of *TNFSF15* secrete more TL1A earlier than haplotype A carriers when stimulated with immune complexes(89). Comparisons between Jewish and non-Jewish haplotype B carriers reveal a higher rate of TL1A secretion amongst Jewish CD patients. Furthermore, if patients are divided based on their seroreactivity to OMPC, monocytes from Jewish OMPC+ and OMPC- haplotype B carriers secrete significantly more TL1A compared to haplotype A carriers when stimulated with immune complexes. The higher basal expression and rapid kinetics of TL1A in Jewish haplotype B carriers suggest a greater TL1A-dependent T cell activation which correlates with the increased severity of disease seen phenotypically (83). This evidence strongly supports *TNFSF15* as a CD severity gene.

TL1A can be membrane-bound or soluble in form and acts as a ligand for DR3, which is mainly expressed on the surface of lymphocytes(90). Baseline DR3 expression occurs in a small subpopulation of CD4+ T cells however its expression is markedly upregulated in CD4+ T cells activated with an anti-CD3 antibody(91). TL1A's interaction with DR3 provides a co-stimulatory signal that results in the activation and proliferation of T cells and secretion of IFN-gamma(92, 93). TL1A's role in the Th1 immune response is highlighted by studies showing increased TL1A expression in the small intestinal and colonic mucosa of patients with CD, a predominantly T helper(Th) 1 driven inflammatory disease(92, 94, 95).

Stimulation of PBMC by TL1A or an agonistic DR3 antibody dose-dependently augments production of IFN-gamma and not IL-4 or IL-10. Although this increase occurs independently of IL-12 and IL-18, TL1A synergizes with IL-12 and IL-18 to enhance IFN-gamma production(95). TL1A also augments IFN-gamma synthesis by IL-12/IL-18 primed CCR9+CD4+ peripheral blood (PB) T cells, a subset of T cells involved in the inflammatory immune response in the small intestine(96, 97). CCR9+ T cells mainly exist in the intestinal immune compartment and are enriched in the peripheral blood of patients with small bowel CD (98-100).

Induction of TL1A expression occurs through a variety of innate immune pathways including activation of Fc gamma receptors, TLRs and NLRs. Microbial activation of TL1A expression occurs in monocytes and DC stimulated with gram-positive, gram-negative, microaerophilic and obligate anaerobes. Furthermore, *E. Coli*-stimulated monocytes co-cultured with IL-12/IL-18 primed CD4+ T cells enhance IFN-gamma production in a TL1A-dependent fashion as neutralizing antibodies to TL1A reduce IFN-gamma levels(101). Activation of pattern recognition receptors using lipopolysaccharide (TLR4), Pam3CSK4 (TLR2), ODN2006 (TLR9) and MDP (NOD2) also induce low levels of TL1A expression(85, 93, 101-104). These findings highlight the importance of the microbial-host interaction in the induction of TL1A and the activation of Th1 responses.

Signaling through the Fc gamma receptor is a potent activator of both membrane-bound and soluble TL1A production in monocytes and monocyte-derived DC isolated from peripheral blood. Production of IFN-gamma by Fc gamma receptor signaling in monocyte and T cell co-cultures is abrogated by an antibody to TL1A(93). Using an engineered construct that expresses a non-cleavable form of membrane-bound TL1A (TL1A-M), TL1A-M binds to DR3 and enhances IFN-gamma expression in IL-12/IL-18 primed CD4+ T cells. This enhancement is 50% of that seen with the full TL1A that produces both the membrane-bound and soluble forms after cleavage(105).

In DSS mouse models of chronic colitis, TL1A enhances Th1 and Th17 effector function by upregulating IFN-gamma and IL-17 production, respectively, in gut-associated lymphoid tissue (GALT) CD4+ T cells under Th1/Th17 polarizing conditions(106). Treatment with an anti-TL1A antibody not only decreases the amount of IFN-gamma and IL-17A produced by GALT, but it attenuates weight loss, colon shortening and the histologic inflammation and injury classically seen in DSS-induced chronic colitis. These findings are corroborated in the G protein alpha i2 knockout T cell transfer model, a model of T cell-mediated chronic colitis(106). This data suggest that TL1A is an important modulator in the development of gut mucosal inflammation.

The effect of TL1A on the induction of Th1 and Th17 cytokines is further investigated using T cells and macrophages isolated from the intestinal LP and peripheral blood of patients with CD. LP CD14+ macrophages are a significant source of TL1A and IL-23 when stimulated by commensal bacteria (103). The addition of soluble TL1A to LP and PB CD4+ T cells induces IFN-gamma production and IL-17A production by LP and not PB CD4+ T cells. Furthermore, soluble TL1A synergizes with IL-23 to enhance even greater production of IFN-gamma and IL-17A by LP CD4+ T cells(103). This data suggest that intestinal macrophages play an important role in the local induction of inflammatory cytokines by TL1A and that TL1A induces cytokines that reflect a Th1 and Th17 immune response.

The role TL1A plays in promoting a Th1 and Th17 response is complex as recent data indicate that TL1A may act as a differential regulator in generation of Th17 cells from naïve T cells. In isolated naïve CD4+ T cells from peripheral blood of healthy donors, activation using anti-CD3/antiCD-28 antibody along with co-stimulation with TL1A results in reduced expansion of CD4+ IL-17 producing cells and IL-17A production(91). TL1A stimulated DR3 deficient mice exhibit an increased number of Th17 cells in inguinal lymph nodes compared to wild-type mice. Furthermore, unlike DR3 knockouts, splenic T cells isolated from wild-type mice stimulated with TL1A results in the inhibition of expansion of IL-17 producing CD4+ T cells(91). The effect of TL1A on *de novo* Th17 differentiation was investigated using naïve

CD4+ T cells exposed to TGF-beta and IL-6. TGF-beta and IL-6 induce differentiation of naïve T cells into Th17 cells (107-109). The addition of TL1A to naïve CD4+ T cells exposed to IL-6 and TGF-beta results in a decreased number of Th17 cells. This decrease occurs independently of STAT1, as inhibition of Th17 cell expansion persists in T cells isolated from STAT1 knockout mice stimulated with TL1A(91). Collectively, these results indicate that TL1A acts to inhibit Th17 expansion and production of IL-17A. One explanation for this is that TL1A differentially affects naïve T cells and T cells committed to a Th17 lineage. Previous studies by our group and others show that TL1A enhances Th17 effector function through production of IL-17A by activated CD4+ T cells(103, 106). These activated T cells may already be committed to Th17 differentiation and thus stimulation by TL1A results in the production of Th17 dependent cytokines. Stimulation of naïve T cells with TL1A under Th17 polarizing conditions inhibits expansion of Th17 cells suggesting that TL1A does not promote *de novo* differentiation of Th17 cells.

DR3, encoded by the *TNFRSF25* gene, is the receptor for TL1A. Signaling through DR3 is not required for differentiation of naïve T cells into Th1, Th2 or Th17 cells(102). DR3 is highly expressed by T regulatory cells (Tregs) and to a lesser extent CD4+FoxP3- conventional T cells (Tconvs)(110). Injection of an agonistic antibody to DR3 into Foxp3 reporter mice results in expansion of Tregs *in vivo* and not *in vitro*. Experiments using MHC-II-deficient (Cd74knockout) or Cd4 knockout mice adoptively transferred with CD4+Foxp3+ cells reveal that MHC-II molecules and T cell receptor signaling are needed for DR3-dependent expansion of Tregs *in vivo*. Furthermore, IL-2 and T cell receptor signaling are required for DR3-induced expansion of Tregs *in vivo* as determined by experiments in Cd80 and Cd86 knockout mice and mice expressing a thymic-targeted transgenic IL-2 receptor beta chain in IL2rb knockouts. DR3 activation, T cell receptor stimulation and IL-2 signaling, however, do not induce expansion of Tregs *in vitro*. The discrepancy in signaling requirements between the *in vitro* and *in vivo* models is attributed to additional unidentified signals that exist in the *in vivo* and not the *in vitro* model(110). TL1A induced expansion of Tregs is further supported by a mouse model of allergic lung inflammation (OVA/alum primed mice followed by OVA airway challenge). In this model, activation of DR3 with an agonistic antibody prior to antigenic exposure results in an increased number of Tregs, a decreased ratio of Tconv/Treg, and decreased mRNA expression of IL-4, IL-5, and IL-13 within the lungs. Lung histology also reveals decreased lymphocytic infiltration and airway mucus production in mice treated with the DR3 agonistic antibody(110). This study highlights a new role for TL1A in the expansion of regulatory T cells.

Sustained expression of TL1A modulates responses by both effector (Teff) and regulatory T cells (Tregs). Overexpression of TL1A by T cells (CD2-TL1A transgenics)(84, 86) and myeloid cells(85, 86) in transgenic mice results in increased production of IFN-gamma(84, 86), IL-13(84, 85) and IL-17(84-86). Furthermore, T cell and myeloid TL1A transgenics exhibit increased number of small intestinal goblet cells, Paneth cells(85, 86) and inflammatory cells(84, 86) in addition to histologic evidence of fibrosis(86). These mice lack colonic inflammation(84-86) but exhibit increased fibrosis in the mucosa and submucosa(86). Examination of T cell subsets within the spleens and lymph nodes of DC TL1A transgenic mice show greater numbers of CD69+, $CD44^{high}$ and $CD62^{low}$ CD4+ T cells compared to controls, suggesting that TL1A enhances activation of conventional T cells(85). The increase in IL-13 and IL-17 gene expression accompanied by increased numbers of IL-13 and IL-17 producing cells isolated from the small intestinal LP of the DC TL1A transgenic mice implicates these CD4+ T cells as a potential source of these interleukins(85). Treatment with anti-IL-13 antibodies reverses the small bowel pathology in the transgenic mice whereas antibodies to IL-17 have little effect on the small intestinal inflammation(84). These studies introduce IL-13 as a prominent cytokine in the role of small intestinal pathology in transgenic mice over-expressing TL1A(84, 85).

Differential expression of TL1A can affect the location of gastrointestinal inflammation. In contrast to lymphoid and myeloid TL1A transgenics that develop small bowel disease and lack colonic inflammation with overexpression of TL1A(84-86), C57BL/10 mice treated with TNBS develop acute colitis(84). Treatment with an antagonistic antibody to TL1A or DR3-Fc, or an antagonistic Fab antibody to DR3 protects these mice from developing microscopic inflammation and weight loss despite no change in the numbers of regulatory T cells in the colon(84). These findings suggest that TL1A's effect on T cells can produce a distinct pattern of inflammation. In the TNBS model of colitis, induction of TL1A results in colonic inflammation while overexpression in myeloid and lymphoid TL1A transgenics leads primarily to small bowel pathology(84). Furthermore, myeloid and lymphoid TL1A transgenic mice develop extra-intestinal pathology including an ulcerated skin lesion and arthropathy at low frequencies(86).

There is increased expansion of Tregs in the secondary lymphoid organs and the LP of DC(85) and T cell(84, 86) TL1A transgenics. Experiments using co-cultures of regulatory and effector T cells isolated from wild-type or DR3 knockout mice suggest that TL1A attenuates suppression of Tregs(85). Addition of TL1A to co-cultures of wild-type Teff and Tregs results in expansion of Teff. However, stimulation with TL1A in co-cultures containing wild-type Teff and DR3 deficient Tregs results in reduced expansion of Teff indicating that intact TL1A signaling through DR3 acts on Tregs to attenuate their suppressive function. Furthermore, stimulation of DR3 deficient Teff co-cultured with wild-type Tregs with TL1A results in reduced expansion of Teff suggesting the TL1A also plays an important role in the expansion of Teff. Lastly, TL1A completely inhibits expansion of Teff in co-cultures of DR3 deficient Teff and Tregs. This data highlight TL1A's role in the expansion of Teff and in the attenuation of Treg suppressive function(85).

Collectively, this data suggest a critical role of TL1A in modulating the Th1 and Th17 response through

the production of IFN-gamma and IL-17. Furthermore, TL1A is shown to influence the T regulatory cell response through increased expansion of these cells. There is accumulating evidence indicating that although there are increased numbers of T regulatory cells, TL1A acts upon these cells to reduce their ability to suppress the expansion of activated T cells. The ability of TL1A to enhance effector T cell function through production of inflammatory cytokines and to inhibit regulatory T cell function makes it an ideal therapeutic target. The importance of TL1A as a potential therapeutic target is validated by the fact that TL1A antibodies attenuate gastrointestinal inflammation in multiple mouse models of acute and chronic colitis.

8. ALTERATIONS IN ADAPTIVE IMMUNITY

The adaptive immune response consists of B and T lymphocytes, recognizes and generates specific responses to foreign antigens or infected cells and is responsible for immunologic memory. CD appears to result from Th1 production of IL-12, IFN-gamma, and TNF-alpha and Th17 production of IL-17 and IL-23. UC however, is predominantly a Th2 and NK T cell disease and results in the production of IL-4, IL-13 and IL-5(83). Human studies and murine models of IBD highlight the importance of CD4+ T cells in the production of inflammatory cytokines and development of mucosal inflammation. CD4+ T cells isolated from patients with CD produce more IFN-gamma and IL-17 compared to UC patients and controls(111). The increased production of IFN-gamma occurs in a Th1-dependent fashion as there is increased T-bet and RORgamma expression. Furthermore, DC isolated from CD patients stimulated with bacterial antigens produce more IL-23 compared to UC patients and controls(111). Understanding the biological alterations underlying the dysregulated adaptive immune response in CD that drives production of IL-23, IL-17 and IL-12 may identify novel therapeutic targets in treatment of patients with IBD.

8.1. Th17 cells and the IL-23/IL-17 axis

Th17 cells are a subgroup of helper T cells that differentiate to TGF-beta and IL-6 and the transcription factors, RORgammaT and RORalpha(107-109, 112). IL-23 produced by APC induces expansion of Th17 cells but is not required for their initial differentiation, as naïve T cells do not express the IL-23R(109, 113, 114). GWAS have identified several genes involved in Th17 differentiation, including *IL-23R*, *IL-12B*, *JAK2*, *STAT3*, *CCR6* and *TNFSF15*, as CD susceptibility genes with some overlap with UC(10, 28). Activated Th17 cells mediate its effector function by its production of IL-17A, IL-17F, IL-21 and IL-22(115, 116).

IL-23 is a member of the IL-12 cytokine family and consists of a heterodimer of the p40 subunit of IL-12 and a unique p19 subunit(117). There is increased expression of IL-23 by LP macrophages isolated from patients with CD(118) implicating IL-23 as an important cytokine in the development of colitis.

Studies of IL-23 in various mouse models of colitis provide further evidence to support its role in the development of mucosal inflammation. Experiments using mice infected with *Helicobacter hepaticus* (*H. hepaticus*) or adoptive transfer of $CD45RB^{hi}$ T cells reveals up-regulation of IFN-gamma, IL-23 and IL-17 in inflamed colonic tissue(119-121). Furthermore, when IL-10 deficient mice are crossed with mice lacking the subunits IL-12p35 or IL-23p19, it is IL-23 and not IL-12 that is needed to promote chronic mucosal inflammation(120). The finding that administration of IL-23 exacerbates colitis in RAG mice adoptively transferred with naïve T cells and increases IL-6 and IL-17 mRNA expression in $CD4+CD45RB^{low}$ memory T cells isolated from IL-10 knockout mice further illustrates the relevance of IL-23 in intestinal inflammation(120). Recent data show that IL-23 is not only involved in T cell driven mucosal inflammation but also exerts inflammatory effects on innate immune cells. The development of mucosal inflammation in the innate immune colitis model of Rag knockout mice infected with *H. hepaticus* is IL-23 dependent(121). Increased expression of IL-17, IL-22 and IFN-gamma occurs in LP cells and not spleen cells isolated from this model suggesting that the sources of these cytokines are innate immune cells. Stimulation of these LP cells with IL-23 results in secretion of IL-17, IL-22 and IFN-gamma confirming this hypothesis. The cells producing IL-17 and IFN-gamma were identified to be novel innate lymphoid cells that express Thy-1, stem cell antigen 1(SCA-1) and IL-23R. This data collectively highlights the importance of IL-23 both in the innate and adaptive phase of mucosal inflammation(122).

Targeted treatments against IL-23 include an anti-p40 antibody (antibody against p40 subunits of IL-12 and IL-23). In patients with active CD, two trials using an anti-p40 antibody demonstrate higher response rates defined as a reduction in CDAI within the induction phase of treatment compared to placebo(123, 124). These response rates were significantly lower compared to trials done in patients with psoriasis(125, 126), which may be attributable to pharmacokinetics and diversity in the biological alterations that underlie the pathogenesis of CD in those individuals.

IL-17 is a member of the IL-17 family of six cytokines along with IL-17F. It signals through the IL-17 receptor A (IL17RA) and acts through activation of the NF-KB and MAPK pathways(127, 128). The genes encoding IL-17 and IL-17F are located on chromosome 6, and these cytokines are produced by Th17 cells that proliferate in response to IL-23(113). Expression of IL-17 is associated with numerous inflammatory diseases including rheumatoid arthritis, asthma, CD and UC(129-131).

IL-17 may differentially exert a pathogenic or protective effect depending on the experimental model in which it is studied. IL-17-deficient mice or those treated with neutralizing antibodies to IL-17 or its receptor are resistant to the development of rheumatoid arthritis and EAE in murine models(113, 132-134). Similarly, studies in IL-17RA knockout mice treated with TNBS reveal that IL-17 is essential in the development of acute mucosal inflammation(135). However, since both IL-17A and IL-17F signal through the IL-17RA it is unclear which cytokine actually contributes to the development of colitis.

In the DSS colitis model, colitis is ameliorated and exacerbated by IL-17F and IL-17A deficiency, respectively, suggesting an important role of IL-17F in the development of mucosal inflammation in this model (130, 136). Using the adoptive transfer model, transfer of IL-17A or IL-17R knockout CD45RB^{hi} T cells into Rag deficient mice causes a severe wasting disease characterized by accelerated weight loss and enhanced Th1 expression with increased expression of IFN-gamma, osteopontin and T-bet (137). The addition of recombinant IL-17 reverses the expression of Th1 markers in mice receiving the IL-17A knockout CD45RB^{hi} T cells suggesting that IL-17A acts to protect against Th1 activation(137). These initial studies suggest that IL-17A may protect against the development of mucosal inflammation whereas IL-17F may drive it.

8.2. IL-12

IL-12, a heterodimer composed of a p40 and p35 subunit, is secreted by activated myeloid cells(138). It is responsible for Th1 maturation and secretion of IFN-gamma and TNF-alpha both by NK cells and T cells(139). Binding of IL-12 to its receptor initiates a cascade of events that lead to activation and nuclear translocation of STAT4 with promotion of Th1 responses (140). Th1 cells are characterized by their transcription factor T-bet and play a role in chronic inflammatory conditions (141-143). GWAS show that variants in the *IL-12B* gene are associated with CD(10). Furthermore, increased expression of IL-12 is noted in the lamina propria mononuclear cells (LPMC) of patients with CD(144). Elevated expression of IL-12 is seen in several models of colitis including DSS, TNBS and G protein alpha i2-deficient mice(145-147).

Initiation of adaptive immunity requires interactions between activated APC and antigen specific T cells. This contact between T cells and APC is coined the immunological synapse (IS)(148, 149). The formation of the IS includes polarization of the T cell by reorientation of the T cell microtubule organizing center (MTOC) and the microtubule cytoskeleton toward the APC (150). This allows for secretion of lymphokines and lytic granules towards the APC(150-152). Interactions between CD40 and its ligand CD154 are important in the cross talk between DC and T cells(153-155). The recruitment of CD154 to the IS is shown to be dependent on T cell polarity using the inhibitor colchicine (colx) and an inhibitor of atypical protein kinase Cs (PKCs). Furthermore, secretion of IL-12 by DC requires T cell polarity as T cells treated with colx induce less IL-12 production by DC compared to untreated T cells(150).

Although the IL-12 secreted by myeloid cells drives naïve T cells towards a Th1 phenotype, recent data suggest that IL-12 may be involved in the development of Th1/Th17 cells which exhibit features of both Th1 and Th17 cells and produce IFN-gamma and IL-17(156). These Th1/Th17 cells are thought to be generated from "unstable" Th17 cells in an intermediate differentiated state(136). Traditionally, *in vitro* studies show that generation of Th1 and Th17 cells are mutually exclusive given that TGF-beta blocks induction of IFN-gamma and T-bet and IFN-gamma inhibits generation of Th17 cells from naïve T cells(157, 158). Th1/Th17 cells can be generated from Th17 cells by exposure to IFN-gamma and IL-12(156). *Ex vivo* isolated Th17 cells that produce IL-17 lack IL-12Rbeta-2 and are not IL-12 responsive. However, upon stimulation with IFN-gamma, IL-12Rbeta-2 is up-regulated, which restores IL-12 responsiveness. Moreover, stimulation with IFN-gamma and IL-12 do not affect the expression of ROR-gammaT, and these Th1/Th17 cells co-express T-bet and ROR-gammaT. This study helps elucidate the molecular mechanism by which a new subset of helper T cells is generated (156).

9. MOLECULAR PATHWAY INTEGRATION

The identification of IBD susceptibility genes is just the beginning in comprehending the pathophysiology of IBD. Given that IBD is a complex polygenic disease and each susceptibility gene only has modest effects on the development of disease, it is possible that interactions between genes affect the risk of developing disease. Determining the functionality of these susceptibility gene products and how they interact in molecular pathways to cause a dysregulated immune response is critical in understanding the inflammatory disease process and in developing novel therapeutic agents.

9.1. Gene - gene interactions

9.1.1. TLR8 and TL1A

Genes that encode TL1A and TLR8 are susceptibility genes in the development of CD and variants in each gene contain both risk and protective haplotypes(57, 89). TL1A appears to be an important regulatory cytokine, and it may play a significant role in modulating the transition from the innate to adaptive immune response.

Activation of TLR8 by its ligand R848 in human monocytes leads to production of the pro-inflammatory cytokines IL-6 and TNF-alpha and the anti-inflammatory cytokine, IL-10, but inhibits Fc gamma induction of TL1A mRNA and protein expression. In addition, activation of TLR8 signaling prevents the production of IFN-gamma from CD4+ T cells(159). The ability of TLR8 signaling to induce pro-inflammatory and anti-inflammatory cytokines hints at TLR8's role in the modulation of inflammation. This role is further supported by the fact that certain haplotypes within the *TLR8* gene confer risk or protection. The ability of TLR8 signaling to inhibit TL1A expression may make TLR8 a potential therapeutic target in the treatment of CD.

9.1.2. NOD2 and ATG16L1

Interactions between NOD1, NOD2 and ATG16L1 play critical roles in the innate immune response. There is accumulating evidence suggesting that alterations in *NOD2* and *ATG16L1*, two different CD susceptibility genes, interact to affect microbial handling(160). Transfection of HeLA cells with tagged NOD1, NOD2, ATG5 and ATG16L1 reveals that a fraction of NOD1, NOD2 and ATG16L1 is present at the plasma

membrane with NOD1 and NOD2 co-localizing with ATG16L1 at the cell surface. Furthermore, interactions between NOD proteins and ATG16L1 are important for the sequestration of bacteria into autophagosomes as NOD1 or NOD2 co-localize with ATG16L1 at the site of entry of *Shigella flexneri* (*S. flexneri*) into the cell. The L1007fs mutation in NOD2, the most prevalent NOD2 mutation in CD, is not able to recruit ATG16L1 to the plasma membrane. When infected with *S. flexneri*, HeLa cells expressing this NOD2 mutation do not exhibit interactions between NOD2 and ATG16L1 at the membrane site of bacterial entry(160).

Using primary human macrophages, DC and the human colonic epithelial cell line HCT116, MDP stimulation activates autophagy and enhances clearance of intracellular *Salmonella typhimurium* (*S. typhimurium*) infection(161). This enhancement depends on autophagy and NOD2 since RNAi knockdown of ATG16L1 expression and transfection of NOD2 RNAi blocks intracellular killing of *S. typhimurium*. Transfection of HEK293T cells (a cell line derived from human embryonic kidney cells) with expression vectors for three major NOD2 mutants, R702W, G908R and L1007fs, impairs NF-KB signaling and clearance of *S. typhimurium* infection. HEK293T cells expressing the L1007fs NOD2 also lack the ability to make mature autophagosomes, evidenced by lack of changes in LC3 levels. Furthermore, inhibition of the first stage of autophagy by a phosphatidylinositol 3-kinase complex (PI3 kinase) inhibitor results in dose-dependent inhibition of NOD2 signaling, and RNAi knockdown of ATG16L1 impairs MDP-stimulated NF-KB signaling(161). In contrast to what is found in epithelial cell lines, human monocytes homozygous for the T300A variant of *ATG16L1* have no alterations in autophagy, intracellular clearance of *S. typhimurium* or production of TNF-alpha compared to heterozygous and wild-type individuals(161). This data emphasize that ATG16L1 and NOD2 interact in a common pathway of microbial handling that is altered by variants in these proteins.

NOD2-mediated autophagy is also required in microbial handling and in the development of major histocompatibility complex (MHC) class II CD4+ T cell responses(162). In human DC, stimulation of NOD2 with MDP leads to MHC class II DM localization with LC3 and results in increased expression of MHC class II on the cell surface. DC isolated from CD patients with variants in *NOD2* and *ATG16L1* stimulated with MDP do not exhibit up-regulation of surface MHC class II. This is thought to be due to defects in autophagy as MHC class II DM does not co-localize with LC3. Exposure of DC exhibiting variant *NOD2* to GFP-labeled *S. enterica* or *E. coli* results in a decreased number of bacteria localized within lysosomes compared to controls. Antithetical findings exist with rapamycin (an activator of autophagy) treatment suggesting that *NOD2* variants affect the cells' ability to clear intracellular bacteria via autophagy(162).

These papers highlight the importance of NLRs and autophagy in the clearance of intracellular microbes and provide further evidence to the theory that impaired microbial clearance leads to persistent activation of the innate and adaptive immune responses and the production of inflammatory mediators.

9.2. Environmental – gene interactions

Through GWAS there is a better understanding of the genetic susceptibility of IBD; however, little is known about the environmental triggers. Recent data show how infection with norovirus in *ATG16L1* variant mice produces the development of features of CD. Persistent infection with a murine norovirus strain (MNV-CR6) in mice with hypomorphic ATG16L1 expression and reduced autophagy leads to abnormalities in granule formation and exocytosis in Paneth cells (163). These features are similar to those found in patients homozygous for the CD risk allele *ATG16L1*. Exposure of these mice to a mild concentration of DSS, in the setting of persistent infection with MNV-CR6, results in colonic ulcerations with associated mucosal inflammation, mesenteric stranding, and small intestinal villous atrophy. This inflammatory response depends on TNF-alpha, IFN-gamma, and the commensal intestinal flora, given that blocking antibodies and broad-spectrum antibiotics attenuate or prevent the inflammatory response(163). These data highlight the concept that the development of IBD is a complex, multiple hit process that results from environmental triggers in genetically predisposed individuals.

10. PERSPECTIVE

Advances in current molecular technologies including GWAS and metagenomic sequencing allowed for considerable progress in elucidating the pathophysiology of IBD. The immunologic processes underlying CD and UC were originally thought to be distinct; however, it is now apparent that overlap in susceptibility genes for these diseases exist. IBD is a complex, heterogenous disease that arises from alterations in the microbial environment and the host immune response to this environment. Further understanding of the genetic and molecular changes that underlie IBD may lead to classification of individuals into subgroups according to their genetic and biologic alterations, which will allow for more individually targeted and potentially more successful therapies.

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Abbreviations: IL-X: interleukin-X, NF-KB: nuclear factor-kappa B, TNF-X: tumor necrosis factor-X, TGF-X: transforming growth factor–X, NODX: nucleotide-binding oligomerization domain-containing protein X, CARDX: caspase recruitment domain X, STATX: signal transducer and activator of transcription X, PTGER4: prostaglandin E receptor 4, JAK2: Janus kinase 2, TYK: tyrosine kinase, SMADX: SMAD family member X, TAGAP: T-cell activation RhoGTPase activating protein, VAMPX: vesicle-associated membrane protein X, FcGR2a: Fc fragment of IgG, low affinity IIa receptor, NDIF1P: Nedd4-family interacting protein 1

Key Words: Crohn’s Disease, Ulcerative Colitis, Autophagy, Microbiome, Innate Immunity, Adaptive Immunity, Bacterial-Host Interactions, Review

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