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Rethinking Mechanisms of Autoimmune Pathogenesis

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Abstract

Why exactly some individuals develop autoimmune disorders remains unclear. The broadly accepted paradigm is that genetic susceptibility results in some break in immunological tolerance, may enhance the availability of autoantigens, and may enhance inflammatory responses. Some environmental insults that occur on this background of susceptibility may then contribute to autoimmunity. In this review we discuss some aspects related to inhibitory signaling and rare genetic variants, as well as additional factors that might contribute to autoimmunity including the possible role of clonal somatic mutations, the role of epigenetic events and the contribution of the intestinal microbiome. Genetic susceptibility alleles generally contribute to the loss of immunological tolerance, the increased availability of autoantigens, or an increase in inflammation. Apart from common genetic variants, rare loss-of-function genetic variants may also contribute to the pathogenesis of autoimmunity. Studies of an inhibitory signaling pathway in B cells helped identify a negative regulatory enzyme called sialic acid acetyl esterase. The study of rare genetic variants of this enzyme provides an illustrative example showing the importance of detailed functional analyses of variant alleles and the need to exclude functionally normal common or rare genetic variants from analysis. It has also become clear that pathways that are functionally impacted by either common or rare defective variants can also be more significantly compromised by gene expression changes that may result from epigenetic alterations. Another important and evolving area that has been discussed relates to the role of the intestinal microbiome in influencing helper T cell polarization and the development of autoimmunity.

Keywords

Autoimmunity; epigenetics; genetic variants; microbiome; SIAE

Paul Ehrlich had recognized in the 1890s that the immune system might attack the host it is meant to protect - a phenomenon he called "horror autotoxicus". No clear mechanisms of relevance to autoimmunity were proposed until the early 1950s well before the function of lymphocytes had been elucidated, Ray Owen, Macfarlane Burnet and Peter Medawar made independent contributions that led to the recognition of the phenomenon of immunological tolerance. In the mid 1940s Owen examined genetically different twin calves, often from

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two fathers but the same mother, that had shared a circulation in utero. He noted that individual calves were unable to make immune responses after birth to antigens derived from the twin they had shared a circulation with. Burnet and Frank Fenner interpreted these findings to constitute evidence for immunological tolerance in the second edition of their book *The Production of Antibodies* published in 1949 (1).

Medawar had defined the laws of transplantation in studies on rabbits and mice, but had been unable to understand why skin grafts took in non-identical twin calves. He read Burnet and Fenner's description of Owen's studies and realized that he had in fact been studying the phenomenon of immunological tolerance. With his colleagues Rupert Billingham and Leslie Brent he experimentally demonstrated the induction of immunological tolerance in inbred mice (2). Medawar and Burnet shared the Nobel prize in Medicine and Physiology in 1960. Although tremendous advances have been made in lymphocyte biology and genetics since then, our understanding of the underlying basis for autoimmunity remains incomplete.

Why do some individuals develop autoimmunity? Common wisdom holds that some combination of genetic susceptibility and environmental factors contributes to the development of disease. Current paradigms have been developed by looking at common genetic variants and rare genetic variants and attempts are currently being made to explore the role of the microbiome in disease. We will review approaches to genetic susceptibility largely through the prism of trying to connect genetics to a break in tolerance. We will also examine two alternative possibilities to mechanisms of susceptibility that go beyond the role of inherited genes and the microbiome.

A still evolving story: genetic bases of common autoimmune disorders

It is widely appreciated that twin studies have helped establish that common autoimmune disorders such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus and multiple sclerosis among others must have a genetic basis. Support for a genetic basis for common autoimmune disorders has also been obtained from studies of common genetic variants (polymorphisms) as well as of rare genetic variants. However, although genetic susceptibility is undoubtedly relevant, the degree to which genetic changes can be linked to disease susceptibility is limited. Genome Wide Association Studies have resulted in relatively small Odds Ratios as discussed in more detail below. While rare genetic variants may have stronger effects - validation will require the examination of tens of thousands of subjects in order to achieve statistical significance. This kind of validation has begun to be obtained. There are a few relatively rare "single-gene" autoimmune disorders in which susceptibility alleles are tightly linked to disease.

A large number of human autoimmune disorders involve the production of pathogenic auto-antibodies. Indeed in some autoimmune disorders believed to be primarily linked to defects in immune regulation by T cells, a prominent role for B cells has re-emerged with the advent of therapeutic trials using antibodies to CD20 (3). Relatively rare autoimmune syndromes have been linked to loss of function mutations in single genes such as AIRE, a regulator of gene expression in thymic medullary epithelial cells, and FoxP3, a transcription factor for T regulatory cells (4–7). The role of these genes in relatively common autoimmune disorders

is unclear. Other single gene diseases include the Autoimmune Lymphoproliferative Syndrome linked to loss of function mutations in Fas or Caspase 10, and Omenn syndrome caused by partial loss of function mutations in RAG1, RAG2, Artemis and other genes involved in V(D)J recombination.

“Common variants”, “Rare variants” and autoimmunity

It is widely recognized that most common autoimmune diseases, including systemic disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), represent non-Mendelian polygenic diseases (8–10). Candidate gene approaches as well as genome wide association studies using SNPs have been generally used to identify susceptibility genes. Some progress has been made in identifying non-HLA genes as susceptibility loci in these non-Mendelian polygenic autoimmune disorders.

A small number of polymorphic variants of candidate genes have been found to confer susceptibility to autoimmune diseases. Some have been identified initially using genetic approaches, whereas others represent candidate genes that have been pursued based on their functional roles in innate or adaptive immunity. Genome wide association studies involving a wide array of SNPs (single nucleotide polymorphisms) have begun to reveal valuable information about “common variants” that are linked to autoimmune disease. Although there has been success in identifying linkage to polymorphic variants in the context of human autoimmune disease, the magnitudes of these associations so far have been relatively weak, with Odds Ratios (ORs) ranging from 1.1 to 2.0.

It has been recognized from a theoretical standpoint (11) for some time that while genome wide association studies are well suited for the identification of “common variants”, they would often lack the power to efficiently identify genes in which multiple different allelic variants, so-called “rare variants” may be linked to disease susceptibility. The “rare variant” hypothesis for multigene diseases first found support in a study of multiple allelic variants linked to the predilection for low HDL levels (12). In the context of autoimmunity, rare variants of the TREX1 exonuclease have been linked to lupus (13). However the rare variant hypothesis requires deep re-sequencing and has been more labor intensive and expensive to pursue. In addition, the ability to assess the functionality of all rare variants is required in order to permit a proper interpretation of data on these types of variants. The study on variants linked to HDL levels used theoretical predictive approaches to “guess” at the functionality of individual variant alleles (12).

Known genetic variants and a break in tolerance at the B or T cell level

Few polymorphic loci so far described in human autoimmunity explicitly affect B cell function, although the Sle1 locus in mice influences receptor editing and susceptibility to lupus in rodents in a yet to be explained way (14). Not surprisingly some of the genes that have been found to be linked to human autoimmune diseases may represent negative regulators of immune function. The *PTPN22* gene encodes a protein tyrosine phosphatase that regulates the activity of Src family kinases in T cells. The R620W *PTPN22* allele is linked to RA, type 1 diabetes (T1D), and to autoimmune thyroid disease (AITD) with ORs ranging from 1.5 to 2 (15–17). This R620W *PTPN22* variant results in the defective

clearance of self reactive B cells and thus contributes to autoimmunity (18). A polymorphic variant of the *CTLA4* gene, which encodes an inhibitory receptor of the CD28 family, is linked to a similar spectrum of autoimmune diseases with an OR ranging from 1.15 to 1.5 (19, 20). The *PDI* gene encodes yet another inhibitory receptor of the CD28 family, and a polymorphic variant has been reported to be linked to SLE with an OR of 1.6 (21). Genetic dysregulation of inhibitory signaling represents one mechanism driving autoimmunity (Table I). This review examines the relevance in human autoimmune disorders of a distinct enzymatic regulator of inhibitory receptors in B cells in some detail as an illustrative example.

Inhibitory receptors in B cells linked to autoimmunity: implicating genome and epigenome

Considerable evidence exists to implicate inhibitory receptors in B cells in the regulation of humoral autoimmunity (22–26). The Fc γ RIIb1 molecule inhibits B cell signaling relatively late in the antibody response and contributes to a phenomenon known as antibody feedback. Mice engineered to lack the Fc γ RIIb1 gene develop a lupus like syndrome, presumably because the absence of this inhibitory receptor facilitates the expansion and terminal differentiation of activated self-reactive B cell clones. Lupus prone mice express lower levels of this inhibitory receptor on germinal center B cells and promoter polymorphisms in this gene have been linked to SLE (27–29).

We have been interested in the negative regulation of BCR signaling and its consequences for B cell development (30–33). BCR signal strength is regulated by a small subset of known proteins including an inhibitory receptor on B cells called CD22 (34–39). The loss of CD22, like that of the transcription factor Aiolos, leads to a reduction in MZ B cells and the loss of B cells that we now describe as perisinusoidal B cells in the bone marrow (40). CD22 contains seven extracellular Ig-like domains and an ITIM motif in its cytoplasmic tail. It is a B lineage restricted member of the Siglec family of vertebrate sialic acid binding Ig-like lectins (41–48).

Tyrosine phosphorylation of the ITIM motif on the cytoplasmic tail of CD22, primarily by Lyn, is believed to contribute to the recruitment of the SHP-1 tyrosine phosphatase, and the inhibition of BCR signaling (49–53). Initiation of inhibitory signaling by CD22 is not well understood, but might involve lectin dependent homo-oligomerization and/or carbohydrate-independent association of this Siglec with the BCR (54, 55). An important finding made in recent years was that mutants of CD22 that cannot bind sialic acid are unable to negatively regulate BCR signaling when reintroduced into CD22 null B cells, although wild type CD22 can do so (56). Wild type CD22 recruits SHP-1 whereas the specific Ig domain mutants that cannot bind sialic acid are incapable of recruiting SHP-1. It may thus be inferred that the B cell intrinsic binding “in cis” of CD22 to an α 2,6 sialic acid containing ligand on the outside of the cell (or in the lumen of an intracellular vesicle) may be crucial for cytosolic SHP-1 recruitment and negative signaling. However a knockin mouse carrying a lectin-domain mutation in CD22 displays some but not all the phenotypic features of CD22 null mice (57).

CD22^{-/-} mice generate IgG anti-DNA autoantibodies after 9 months of age, and Lyn deficient mice develop a lupus like disease. CD22 is expressed in mature B cells and it is believed that weakly self-reactive cells are restrained from responding to self-antigen because of a threshold set by inhibitory signals from CD22. CD22 therefore may be a player in peripheral tolerance, but it is worth noting that while CD22^{-/-} mice spontaneously develop anti-DNA antibodies as they age, these mice do NOT develop lupus (23). This point is raised to contrast the failure to develop a full blown lupus in the absence of CD22 with a more severe phenotypic alteration seen in the absence of an enzyme called SIAE (Sialic Acid acetyl esterase) (58).

Peripheral B cells express CD22 at all times and always synthesize and express $\alpha 2,6$ linked sialic acid containing ligands. Why does CD22 not always send inhibitory signals? How exactly CD22 signaling is regulated *in vivo* is poorly understood. We reasoned that a specific modification of sialic acid that serves as an ON/OFF switch for CD22 may represent a potentially powerful way to modulate BCR signaling and CD22 function. It has been shown that 9-*O*-acetylation of sialic acid, wherein the outermost hydroxyl group is modified by a yet to be identified sialyl 9-*O*-acetyl transferase, destroys the ability of Siglecs to recognize their ligands. This hypothesis was the basis that led to our discovery that a specific Sialyl acetyl esterase (SIAE) is a major regulator of B cell development and of peripheral tolerance, not just in knockout mice (58–60), but likely also in individuals with inherited loss-of-function mutations in this gene (61) and more widely in large numbers of individuals with lupus and rheumatoid arthritis in which the SIAE pathway is epigenetically dysregulated (Kendra Taylor and SP unpublished).

Initial studies on human genetic rare variants of *SIAE* revealed the presence of a number of heterozygous catalytically defective rare variants of this gene that are enriched in subjects with autoimmunity (61, 62). No GWAS signal had been observed at the *SIAE* locus. One common variant of *SIAE*, M89V SIAE was erroneously considered to be of relevance because in our initial relatively small analysis it was found in homozygous form in subjects with disease but was seen only in heterozygous form in controls. This variant was NOT catalytically defective. It is clearly found in equivalent frequencies in disease subjects and controls (63, 64) and as a functionally normal allele should have been excluded from analysis (65). When this catalytically normal common variant allele is actually excluded from consideration, catalytically defective rare alleles of *SIAE* are still found to be linked to autoimmunity (63). Concerns had been raised (64) that any relevant locus should generate a signal on GWAS. It is worth noting that the evolutionary history of common genetic variants and rare genetic variants is very different. Common genetic variants likely arose in populations many thousands of years ago. Rare genetic variants are likely of more recent origin and may even in some cases have arisen *de novo*. There is no strong theoretical reason to assume that rare genetic variants and common genetic variants must be physically linked.

It is now emerging that the SIAE pathway likely plays a large role in autoimmunity. This pathway as mentioned above appears to be epigenetically regulated in almost half of all lupus and rheumatoid arthritis disease subjects but not in controls. Catalytically defective rare genetic variants in the *SIAE* gene as well as epigenetic regulation of this inhibitory

pathway should be examined in tens of thousands of subjects with autoimmunity and in controls to accurately gauge its relevance in a range of autoimmune disorders.

Some theoretical considerations about epigenetics and disease

From the autoimmune context gene or protein expression may be altered in either naive B and T cells or in memory lymphocytes sometimes because of an altered cellular milieu that is generated by genetic variation, or it could reflect an altered milieu generated by metabolites released by specific components of the microbiome, or by exposure to environmental chemicals including drugs and xenobiotics. Cell signaling may be altered by microbial products and microbial metabolites may be generated that alter the cellular milieu intra-cellularly. All cells in any given vertebrate contain the same genes but only some are expressed in lymphocytes and immune cells while others are silenced in these cells. Epigenetics refers to mechanisms that control gene expression that go beyond the actual sequence of DNA in individual genes. DNA in chromatin is wound around a protein core of histone octamers, forming structures called nucleosomes, which may be either well separated from other nucleosomes or densely packed. Chromatin may therefore exist as relatively loosely packed structures called euchromatin wherein genes are available and are transcribed, or as very tightly packed structures called heterochromatin in which genes are maintained in a silenced state. The structural organization of portions of chromosomes therefore varies in different cells, making certain genes available for transcription factors to bind to while these very same genes may be unavailable to transcription factors in other cells. The mechanisms that make genes available or unavailable in chromatin are considered to be epigenetic mechanisms. Broadly speaking there are four types of epigenetic mechanisms of relevance although these will not be discussed here in detail. These are the methylation of DNA on certain cytosine residues that generally silences genes, post-translational modifications of the histone tails of nucleosomes (acetylation, methylation, ubiquitinylation etc) that may either render genes active or inactive depending on the histone modified and the nature of the modification, active remodeling of chromatin by protein machines called remodeling complexes that can also either enhance or suppress, and the silencing of gene expression by non-coding RNAs (reviewed in 66).

Micro RNAs (miRNAs) are small endogenous noncoding RNAs that are initially generated in the nucleus as longer primary miRNA transcripts that are processed at this site by an endoribonuclease called Drosha into shorter pre-miRNAs that have a stem loop structure and can be exported into the cytosol. In the cytosol, the pre-miRNA is processed by another endoribonuclease called Dicer into a short double-stranded miRNA about 21 to 22 base pairs in length, one strand of which can be used to pair with a complementary sequence in a number of cellular mRNAs (reviewed in 67). These mRNAs associate with miRNAs and proteins called Argonaute proteins to form complexes known as RISC (RNA-induced silencing complex). If the 6 to 8 base pair miRNA seed sequence is not perfectly complementary to the mRNA, the mRNA is prevented from being translated efficiently. miRNAs may contribute in significant ways to modulating gene and protein expression in immune cells in response to changes in the milieu of these cells. Deletion of Dicer in the T lineage results in a preferential loss of regulatory T cells and the consequent development of an autoimmune phenotype similar to that seen in the absence of FoxP3. These studies in

animals suggest that miRNAs may control tolerance and autoimmunity but studies in humans that prove this are yet to be reported.

Somatic mutations and autoimmunity

In a review a few years ago Chris Goodnow suggested (and Nossal had made a similar suggestion in the past) that given some parallels that exist between autoimmune diseases and lymphomas perhaps it is worth considering that autoimmunity might be driven in part by mutations that are not in the germline but which, like in cancer, are somatic (68). It is becoming increasingly clear that many therapies that are useful in lymphomas are also useful in autoimmunity. However there is growing evidence that autoimmunity is an oligoclonal or pauciclonal disease and not a monoclonal one. In our own studies using Next Gen Sequencing it is clear that disease related clones can be found in autoimmunity that these are not disorders of single clones. Any somatic mutation based model needs to take this into consideration. It is unlikely that the same clones may acquire the same somatic mutations. We would favor a view wherein memory T and B cell clones arise in a similar altered milieu and this alteration in the milieu causes broad epigenetic alterations that drive autoimmunity.

The Microbiome and Autoimmunity

A picture is now emerging as to how intimately, insidiously and ubiquitously microbes and immune cells communicate and how these interactions influence the normal function of the immune system and the generation of disease. About ninety percent of the cells in our bodies are of microbial origin. As a species we inherit a total of about 20, 500 genes, but the microbes within us express about a million different genes. Microbes inhabit our skins, our oral cavities and the intestine, and can also be found in a number of other tissues including the uterine cavity and lungs. Infected monocytes and macrophages traverse the body carrying microbes and their metabolites. A huge load of metabolites - the small molecules generated from the enzymatic protein products of over a million microbial genes interact with the paltry number of protein products of our genes. It is easy to understand how a host nuclear hormone receptor for instance may have evolved to bind a microbial metabolite that regulates its transcriptional activity.

The connections between the Microbiome and the Immune system are now well established (reviewed in 69). A landmark study from the Littman laboratory. revealed that a single microbial species, segmented filamentous bacteria or SFB is required for the generation of Th17 cells and the subsequent development of autoimmunity (70). This very same bacterial species drove the development of Th 17 cell, autoantibodies and autoimmunity in the $K \times B \times N$ model of arthritis in the mouse (71) and the influence of the intestinal microbiota on murine models of autoimmunity has been established in other models as well (72). A role for indigenous *Clostridial* species in the development of murine Tregs has been noted (73). Apart from the established roles for Th17 cells and regulatory T cells (Table II) the microbiota likely influences the development of other polarized T cells but specific microbial players remain to be identified. Alterations in the microbiome have already been reported in patients with Type II diabetes, a disease that is increasingly seen to have an

immune basis, as well as in autoimmune disorders such as type I diabetes and psoriasis and autoimmune-like disorders such as Crohn's disease and ulcerative colitis, that may be driven by an altered immune response to intestinal microbes (74–77; Table III). It is likely that ongoing studies in humans will help establish that specific gut microbial species contribute to the pathogenesis of specific human autoimmune disorders.

How exactly might microbes influence autoimmunity? For many decades much of the focus on microbes centered around the phenomenon of molecular mimicry- basically cross-reactivity with self. More attention is now being paid to microbial metabolites and the possibility that microbial products can influence host cell function. It has been suggested that the most attention should be paid to intracellular microbes because these intracellular microbes are most likely to alter gene and protein expression (78). For instance, the vitamin D receptor, a nuclear hormone receptor that regulates transcription, has been shown to influence the expression of many immune genes. Many intracellular viruses including the Epstein-Barr virus and cytomegalovirus, and bacteria such as *Borrelia burgdorferi* and *Mycobacterium tuberculosis* have been shown to reduce vitamin D receptor expression (reviewed in 78) and thus influence host immunity. This field remains in its infancy. The next decade promises to be particularly exciting in terms of not just identifying specific environmental factors that influence autoimmunity but also in understanding how exactly they contribute to disease.

Conclusions and Final Comments

It is likely that inherited genetic variation involving both common genetic variants and rare variants contribute to the creation of a milieu around immune cells that favors disease. This milieu may also be influenced by metabolites generated by the intestinal microbiota. This overall change in milieu by a combination of genetic and microbial factor likely facilitates the induction of epigenetic alterations either because of the induction or suppression of specific miRNAs or the altered expression of chromatin regulators. These changes presumably contribute, along with specific microbial insults, to the induction of autoimmune disorders (Fig. 2). Finally, this paper is dedicated to Abul Abbas in a special issue of the Journal of Autoimmunity that honors, as it has in the past, distinguished autoimmunologists and themes that reflect critical issues in rheumatology and immunology (79–82). Abul has made striking and unique contributions in teaching and research, many of which have improved patient care. I personally have enjoyed discussing these issues with Abul Abbas over the years. I have been privileged to teach with Abul at Harvard and continue to teach with him around the world, co-author two textbooks with him and I greatly value our longstanding friendship. Abul's depth of knowledge about immunology, his tremendous enthusiasm not just for the subject but for propagating his love for it by his teaching, his natural warmth and his generosity of spirit have influenced me in numerous ways over the decades.

REFERENCES

1. Burnet, FM.; Fenner, F. The Production of Antibodies. 2nd. Melbourne: Macmillan and Co.; 1949.
2. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature. 1953; 172:603–606. [PubMed: 13099277]

3. Martin F, Chan AC. B cell immunobiology in disease: evolving concepts from the clinic. *Annu Rev Immunol.* 2009; 24:467–496. [PubMed: 16551256]
4. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the Aire protein. *Science.* 2002; 298:1395–1401. [PubMed: 12376594]
5. Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet.* 2003; 39:537–545. [PubMed: 12161590]
6. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet.* 2001; 27:18–20. (2001). [PubMed: 11137992]
7. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2003; 299:1057–1061. [PubMed: 12522256]
8. Gregersen PK, Behrens TW. Genetics of autoimmune diseases--disorders of immune homeostasis. *Nat Rev Genet.* 2006; 7:917–928. (2006). [PubMed: 17139323]
9. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genomewide study. *N Engl J Med.* 2007; 357:1199–1209. [PubMed: 17804836]
10. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med.* 2007; 357:977–986. [PubMed: 17804842]
11. Pritchard JK, Cox NJ. The allelic architecture of human disease genes: common disease-common variant...or not? *Hum Mol Genet.* 2002; 11:2417–2423. [PubMed: 12351577]
12. Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, et al. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science.* 2004; 305:869–872. [PubMed: 15297675]
13. Lee-Kirsch MA, Gong M, Chowdhury D, Senenko L, Engel K, et al. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat Genet.* 2007; 39:1065–1067. [PubMed: 17660818]
14. Kumar KR, Li L, Yan M, Bhaskarabhatla M, Mobley AB. Regulation of B cell tolerance by the lupus susceptibility gene Ly108. *Science.* 2006; 312:1665–1669. (2006). [PubMed: 16778059]
15. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat Genet.* 2004; 36:337–338. [PubMed: 15004560]
16. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet.* 2004; 75:330–337. [PubMed: 15208781]
17. Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, et al. Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes.* 2004; 53:3020–3023. [PubMed: 15504986]
18. Menard L, Saadoun D, Isnardi I, Ng YS, Meyers F, Massad C, et al. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J. Clin. Invest.* 2011; 121:3635–3644. [PubMed: 21804190]
19. Ueda H, Howson JM, Esposito L, Heward J, Snook H, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature.* 2003; 423:506–511. [PubMed: 12724780]
20. Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW, et al. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. *Am J Hum Genet.* 2005; 77:1044–1060. [PubMed: 16380915]
21. Prokunina L, Padyukov L, Bennet A, de Faire U, Wiman B, et al. Association of the PD-1.3A allele of the PDCD1 gene in patients with rheumatoid arthritis negative for rheumatoid factor and the shared epitope. *Arthritis Rheum.* 2004; 50:1770–1773. [PubMed: 15188352]

22. Cornall RJ, et al. Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity*. 1998; 8:497–508. [PubMed: 9586639]
23. O'Keefe TL, Williams GT, Batista FD, Neuberger MS. Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. *J Exp Med*. 1999; 189:1307–1313. [PubMed: 10209047]
24. Bolland S, Ravetch JV. Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. *Immunity*. 2000; 13:277–285. [PubMed: 10981970]
25. Yu CC, Mamchak AA, DeFranco AL. Signaling mutations and autoimmunity. *Curr Dir Autoimmun*. 2003; 6:61–88. [PubMed: 12408047]
26. McGaha TL, Sorrentino B, Ravetch JV. Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science*. 2005; 307:590–593. [PubMed: 15681388]
27. Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR, MacAry PA, et al. Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. *Nat Med*. 2005; 11:1056–1058. [PubMed: 16170323]
28. Kono H, Kyogoku C, Suzuki T, Tsuchiya N, Honda H, et al. FcgammaRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. *Hum Mol Genet*. 2005; 14:2881–2892. [PubMed: 16115811]
29. Blank MC, Stefanescu RN, Masuda E, Marti F, King PD, et al. Decreased transcription of the human FCGR2B gene mediated by the -343 G/C promoter polymorphism and association with systemic lupus erythematosus. *Hum Genet*. 2005; 117:220–227. [PubMed: 15895258]
30. Wang JH, Avital N, Cariappa A, Friedrich C, Ikeda T, et al. Aiolos regulates B cell activation and maturation to effector state. *Immunity*. 1998; 9:543–554. [PubMed: 9806640]
31. Cariappa A, Tang M, Parng C, Nebelitskiy E, Carroll M, et al. The follicular versus marginal zone B lymphocyte cell fate decision is regulated by Aiolos, Btk, CD21. *Immunity*. 2001; 14:603–615. [PubMed: 11371362]
32. Cariappa A, Pillai S. Antigen dependent B lymphocyte development. *Curr. Opin. in Immunol*. 2002; 14:241–249. [PubMed: 11869899]
33. Pillai S, Cariappa A, Moran ST. Positive selection and lineage commitment during peripheral B lymphocyte development. *Immunological Reviews*. 2004; 197:206–218. [PubMed: 14962197]
34. Pillai S, Cariappa A, Moran ST. Marginal Zone B Cells. *Annu Rev Immunol*. 2005; 23:161–196. [PubMed: 15771569]
35. O'Keefe TL, Williams GT, Davies SL, Neuberger MS. Hyperresponsive B cells in CD22-deficient mice. *Science*. 1996; 274:798–801. [PubMed: 8864124]
36. Otipoby KL, Andersson KB, Draves KE, Klaus SJ, Farr AG, et al. CD22 regulates thymus-independent responses and the lifespan of B cells. *Nature*. 1996; 384:634–637. [PubMed: 8967951]
37. Sato S, Miller AS, Inaoki M, Bock CB, Jansen P, et al. CD22 is both a positive and negative regulator of B lymphocyte antigen receptor signal transduction: altered signaling in CD22-deficient mice. *Immunity*. 1996; 5:551–562. [PubMed: 8986715]
38. Nitschke L, Carsetti R, Ocker B, Kohler G, Lamers MC. CD22 is a negative regulator of B-cell receptor signalling. *Curr Biol*. 1997; 7:133–143. [PubMed: 9016707]
39. Samardzic T, Marinkovic D, Danzer CP, Gerlach J, Nitschke L, et al. Reduction of marginal zone B cells in CD22-deficient mice. *Eur J Immunol*. 2002; 32:561–567. [PubMed: 11828373]
40. Cariappa A, Mazo IB, Chase C, Shi HN, Liu H, et al. Perisinusoidal B cells in the bone marrow participate in T-independent responses to blood-borne microbes. *Immunity*. 2005; 23:397–407. [PubMed: 16226505]
41. Stamenkovic I, Seed B. The B-cell antigen CD22 mediates monocyte and erythrocyte adhesion. *Nature*. 1990; 345:74–77. [PubMed: 1691828]
42. Cyster JG, Goodnow CC. Tuning antigen receptor signaling by CD22: integrating cues from antigens and the microenvironment. *Immunity*. 1997; 6:509–517. [PubMed: 9175829]
43. Law CL, Sidorenko SP, Clark EA. Regulation of lymphocyte activation by the cell-surface molecule CD22. *Immunol Today*. 1994; 15:442–449. [PubMed: 7945784]

44. Tedder TF, Tuscano J, Sato S, Kehrl JH. CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. *Annu Rev Immunol.* 1997; 15:481–504. [PubMed: 9143697]
45. Sgroi D, Varki A, Braesch-Andersen S, Stamenkovic I. CD22, a B cell-specific immunoglobulin superfamily member, is a sialic acid-binding lectin. *J Biol Chem.* 1993; 268:7011–7018. [PubMed: 8463234]
46. Powell LD, Sgroi D, Sjoberg ER, Stamenkovic I, Varki A. Natural ligands of the B cell adhesion molecule CD22 beta carry N-linked oligosaccharides with alpha-2,6-linked sialic acids that are required for recognition. *J Biol Chem.* 1993; 268:7019–7027. [PubMed: 8463235]
47. Powell LD, Varki A. The oligosaccharide binding specificities of CD22 beta, a sialic acid-specific lectin of B cells. *J Biol Chem.* 1994; 269:10628–10636. [PubMed: 8144652]
48. Powell LD, Jain RK, Matta KL, Sabesan S, Varki A. Characterization of sialyloligosaccharide binding by recombinant soluble and native cell-associated CD22. Evidence for a minimal structural recognition motif and the potential importance of multisite binding. *J Biol Chem.* 1995; 270:7523–7532. [PubMed: 7706299]
49. Schulte RJ, Campbell MA, Fischer WH, Sefton BM. Tyrosine phosphorylation of CD22 during B cell activation. *Science.* 1992; 258:1001–1004. [PubMed: 1279802]
50. Campbell MA, Klinman NR. Phosphotyrosine-dependent association between CD22 and protein tyrosine phosphatase 1C. *Eur J Immunol.* 1995; 25:1573–1579. [PubMed: 7542197]
51. Doody GM, Justement LB, Delibrias CC, Matthews RJ, Lin J, et al. A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. *Science.* 1995; 269:242–244. [PubMed: 7618087]
52. Law CL, Sidorenko SP, Chandran KA, Zhao Z, Shen SH, et al. CD22 associates with protein tyrosine phosphatase 1C, Syk, and phospholipase C-gamma1 upon B cell activation. *J Exp Med.* 1996; 183:547–560. [PubMed: 8627166]
53. Smith KG, Tarlinton DM, Doody GM, Hibbs ML, Fearon DT. Inhibition of the B cell by CD22: a requirement for Lyn. *J Exp Med.* 1998; 187:807–811. [PubMed: 9480991]
54. Han S, Collins BE, Bengtson P, Paulson JC. Homomultimeric complexes of CD22 in B cells revealed by protein-glycan cross-linking. *Nat Chem Biol.* 2005; 1:93–97. [PubMed: 16408005]
55. Collins BE, Smith BA, Bengtson P, Paulson JC. Ablation of CD22 in ligand-deficient mice restores B cell receptor signaling. *Nat Immunol.* 2006; 7:199–206. [PubMed: 16369536]
56. Jin L, McLean PA, Neel BG, Wortis HH. Sialic acid binding domains of CD22 are required for negative regulation of B cell receptor signaling. *J Exp Med.* 2002; 195:1199–1205. [PubMed: 11994425]
57. Poe JC, Fujimoto Y, Hasegawa M, Haas KM, Miller AS, et al. CD22 regulates B lymphocyte function in vivo through both ligand-dependent and ligand-independent mechanisms. *Nat Immunol.* 2005; 5:1078–1087. [PubMed: 15378059]
58. Cariappa A, Takematsu H, Liu H, Diaz S, Haider K, et al. B cell antigen receptor signal strength and peripheral B cell development are regulated by a 9-O-acetyl sialic acid esterase. *J Exp Med.* 2009; 206:125–138. [PubMed: 19103880]
59. Pillai S, Cariappa A, Pirnie SP. Esterases and autoimmunity: the sialic acid acetyltransferase pathway and the regulation of peripheral B cell tolerance. *Trends Immunol.* 2009; 30:488–493. [PubMed: 19766537]
60. Pillai S, Netravali IA, Cariappa A, Mattoo H. Siglecs and immune regulation. *Annu Rev Immunol.* 2012; 30:357–392. [PubMed: 22224769]
61. Surolia I, Pirnie SP, Chellappa V, Taylor KN, Cariappa A, et al. Functionally defective germline variants of sialic acid acetyltransferase in autoimmunity. *Nature.* 2010; 466:243–247. [PubMed: 20555325]
62. Hirschfield GM, Xie G, Lu E, Sun Y, Juran BD, et al. Association of primary biliary cirrhosis with variants in the CLEC16A, SOCS1, SPIB and SIAE immunomodulatory genes. *Genes Immun.* 2012; 13:328–335. [PubMed: 22257840]
63. Szymanski K, Skorka A, Szypowska A, Bednarczuk T, Ploski R. Functionally defective germline variant of sialic acid acetyltransferase (Met89Val) is not associated with type 1 diabetes mellitus and Graves' disease in a Polish population. *Tissue Antigens.* 2011; 78:214–216. [PubMed: 21615338]

64. Hunt KA, Smyth DJ, Balschun T, Ban M, Mistry V, et al. Rare and functional SIAE variants are not associated with autoimmune disease risk in up to 66,924 individuals of European ancestry. *Nat Genet.* 2012; 44:3–5. [PubMed: 22200769]
65. Chellappa V, Taylor KN, Pedrick K, Donado C, Netravali IA, et al. M89V Sialic acid Acetyl Esterase (SIAE) and all other non-synonymous common variants of this gene are catalytically normal. *PLoS One.* 2013; 8:e53453. [PubMed: 23308225]
66. Zhou VW, Goren A, Bernstein BE. Charting histone modifications and the functional organization of mammalian genomes. *Nat. Rev. Genet.* 2011; 12:7–18. [PubMed: 21116306]
67. Xiao C, Rajewsky K. MicroRNA control in the immune system: basic principles. *Cell.* 2009; 136:36–36.
68. Goodnow CC. Multistep pathogenesis of autoimmune disease. *Cell.* 2007; 130:25–35. [PubMed: 17632054]
69. Honda K, Littman DR. The microbiome in infectious disease and inflammation. *Annu Rev. Immunol.* 2012; 30:759–795. [PubMed: 22224764]
70. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell.* 2009; 139:485–498. [PubMed: 19836068]
71. Wu H-J, Ivanov II, Darce J, Hattori K, Shima T, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity.* 2010; 32:815–827. [PubMed: 20620945]
72. Wen L, Ley RE, Volchkov PY, Starnages PB, Avanesyan L, et al. Innate immunity and intestinal microbiota in the development of type 1 diabetes. *Nature.* 2008; 455:1109–1113.
73. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science.* 2010; 331:337–341. [PubMed: 21205640]
74. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PloS one.* 2010; 5:e9085. [PubMed: 20140211]
75. Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, et al. Toward defining the autoimmune microbiome for type 1 diabetes. *The ISME journal.* 2011; 5:82–91. [PubMed: 20613793]
76. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome biology.* 2012; 13:R79. (2012). [PubMed: 23013615]
77. Gao Z, Tseng CH, Strober BE, Pei Z, Blaser MJ. Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. *PloS one.* 2008; 3:e2719. [PubMed: 18648509]
78. Proal AD, Albert PJ, Marshall TG. The human microbiome and autoimmunity. *Curr. Opin. Rheumatol.* 2013; 25:234–240. [PubMed: 23370376]
79. Selmi C, Leung PS, Sherr DH, Diaz M, Nyland JF, Monestier M, Rose NR, Gershwin ME. Mechanisms of environmental influence on human autoimmunity: a National Institute of Environmental Health Sciences expert panel workshop. *J. Autoimmun.* 2012; 39:272–284. [PubMed: 22749494]
80. Gershwin ME, Shoenfeld Y, Chella David: a lifetime contribution in translational immunology. *J. Autoimmun.* 2011; 37:59–62. [PubMed: 21621385]
81. Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjogren's syndrome: what we know and what we should learn. *J. Autoimmun.* 2012; 39:4–8. [PubMed: 22326205]
82. Jamin C, Renaudineau Y, Pers JO, Pierre Youinou: when intuition and determination meet autoimmunity. *J. Autoimmun.* 2012; 39:117–120.

Research Highlights

- Genetics provides incomplete explanations for autoimmune pathogenesis
- Genetics and environmental alterations may induce epigenetic alterations
- Epigenetic alterations in Immune cells may contribute to autoimmunity
- Altered microbial communities may also contribute to autoimmunity

Fig 1.

The SIAE/Siglec pathway. This pathway regulates peripheral B cell tolerance as described in the text. Glycoproteins including membrane immunoglobulins can be acetylated on the 9-OH position of sialic acid in the late Golgi by a sialic acid acetyl transferase (SIAT) and subsequently de-acetylated by a sialic acid acetyl esterase (SIAE). Loss of function mutations in *SIAE* and more commonly epigenetic inhibition of expression of this enzyme may contribute to the loss of B cell tolerance and the induction of autoimmunity.

Fig 2.

A model integrating genetics, microbiota and epigenetics in the pathogenesis of autoimmunity. Common genetic variants and rare genetic variants may cause alterations in the functions of certain genes that contribute to changes in the internal or external milieu of immune cells. Alteration of cytokine levels for instance could change the external milieu while changes in levels of intracellular metabolites may change the internal milieu. Similarly changes in the microbiome or in exposure chemical toxins or drugs could result either in altered stimulation of immune cells, or the production of unique metabolites that potentially alter the intracellular milieu in immune cells. These changes in the milieu of immune cells could contribute to the altered levels of expression of certain immune cell genes, either by inducing or inhibit certain microRNAs or by regulating the expression of chromatin modifying enzymes. These "epigenetic" changes in gene expression in immune cells caused by the microbiome or by genetic alterations or both could alter immune cell function causing these cells to change the expression of proteins in a way that results in a break in tolerance, or the increased availability of a self-antigen, or an increased ability to induce inflammation. Such changes could contribute to the development of autoimmunity.

Table I**Inhibitory Receptor Pathway Genetic Variation and Autoimmunity**

Gene	Reference
<i>PTPN22</i>	15–18, 20
<i>CTLA-4</i>	19, 20
<i>PD-1</i>	21
<i>FcγRIIB</i>	24
<i>SIAE</i>	61, 62, 65

Table II

Specific Microbiota Changes in mice linked to polarized T cell development

Microbial species	Type of Immune Cell	Reference
Segmented Filamentous Bacteria	Th17 cells	70, 71
<i>Clostridial</i> species	regulatory T cells	73

Table III

Human Autoimmune/inflammatory Diseases Linked to Microbiota alterations

Disease	Reference
Type II diabetes	74
Type I diabetes	75
Psoriasis	77
Crohn's disease	76
Ulcerative colitis	76