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Genomics of lymphoid malignancies reveal major activation pathways in lymphocytes

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ABSTRACT

Breakdown of tolerance leads to autoimmunity due to emergence of autoreactive T or B cell clones. Autoimmune diseases predispose to lymphoid malignancies and lymphoid malignancies, conversely, can manifest as autoimmune diseases. While it has been clear for a long time that a competitive advantage and uncontrolled growth of lymphocytes contribute to the pathogenesis of both lymphoid malignancies as well as autoimmune diseases, the overlap of the underlying mechanisms has been less well described. Next generation sequencing has led to massive expansion of the available genomic data in many diseases over the last five years. These data allow for comparison of the molecular pathogenesis between autoimmune diseases and lymphoid malignancies. Here, we review the similarities between autoimmune diseases and lymphoid malignancies: 1) Both, autoimmune diseases and lymphoid malignancies are characterized by activation of the same T and B cell signaling pathways, and dysregulation of these pathways can occur through genetic or epigenetic events. 2) In both scenarios, clonal and subclonal evolution of lymphocytes contribute to disease. 3) Development of both diseases not only depends on T or B cell intrinsic factors, such as germline or somatic mutations, but also on environmental factors. These include infections, the presence of other immune cells in the microenvironment, and the cytokine milieu. A better mechanistic understanding of the parallels between lymphomagenesis and autoimmunity may help the development of precision treatment strategies with rationally designed therapeutic agents.

1. Introduction

Tolerance of the immune system is tightly regulated. Multiple checkpoints exist with a high degree of redundancy. In the case of $CD4^+$ T cells, thymic deletion, induction of anergy, and the generation of regulatory T cells all cooperate to prevent the outgrowth of potentially autoreactive clones [1]. This redundancy accounts for the fact that clonal expansion and effector function of B and T cells are only temporary and spatially limited, when they occur in the context of pathogen defense. Nevertheless, in some cases, breakdown of one or more of these tolerance mechanisms leads to autoimmune disease. While some autoimmune diseases can be linked to a defect in a single specific gene, such as in the human immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), and the autoimmune lymphoproliferative syndrome (ALPS) with defects in *FOXP3*, *AIRE*, and *FAS*, respectively [2–5], the pathogenesis of the majority of autoimmune diseases is more complex, and most appear to be dependent on defects in more than one gene. It has also been known for a long time that there is a link between autoimmune and inflammatory disorders and malignant lymphomas. These diseases include rheumatoid arthritis (RA), Sjögren's syndrome, systemic lupus erythematosus (SLE), celiac disease and Hashimoto's thyroiditis [6–10]. Some autoimmune diseases may in fact be the early clinical manifestation of an emerging malignancy. For example, the occurrence of autoimmune hemolytic anemia (AIHA) may precede the diagnosis of chronic lymphocytic leukemia (CLL) [7]. Conversely, CLL and other lymphoid neoplasms are often associated with various autoimmune events [11,12].

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From a clinical perspective, autoimmune diseases and lymphoid malignancies may share several characteristics: 1) Excessive proliferation of an autoreactive clone or malignant clone, respectively, 2) inappropriate secretion of cytokines, 3) uncontrolled secretion of antibodies. Antibody secretion in lymphoid neoplasms can result in autoimmunity, or can lead to direct damage through antibody deposition as in multiple myeloma (MM) [13]. Considering the overlap in mechanism and phenotype, one might also expect genetic overlap between lymphoid neoplasms and autoimmunity. It has been proposed that multiple mutations, inherited and somatic, may be needed before a self-reactive clone bypasses sequential tolerance checkpoints, and this may lead to the emergence of autoimmune disease [14]. The genomics of various lymphoid malignancies have recently been characterized by deep sequencing, and these studies have revealed the genetic defects, which underlie their pathogenesis. Since the first complete description of the human genome over 10 years ago [15,16], next generation sequencing has come a long way, and the use of these genomic tools now allows for comparison between the causal defects that lead to the evolution of lymphoid malignancies or the emergence of autoimmunity.

2. Significantly mutated lymphoma genes in B cell activation

Two of the key pathways that are responsible for B cell activation are the B cell receptor pathway and the TLR/MYD88/NFκB pathway. Diffuse large B cell lymphoma (DLBCL) is an aggressive B cell neoplasm that can only be cured in about 60% of cases with multi-agent chemotherapy [17]. Four independent studies have utilized next generation sequencing to determine the most significantly mutated genes in DLBCL [18–21]. In line with previously published functional data, significantly mutated genes often involve genes in the NFκB pathway, including *MYD88*, *CARD11*, *NFKBIA*. *MYD88* has been shown to play a functional role in DLBCL [22]. The L265P mutation of *MYD88* is a gain-of-function driver mutation that is important for the pathogenesis of the activated B cell-like (ABC) subtype of DLBCL, by promoting cell survival through assembly of a protein complex containing IRAK1 and IRAK4, leading to IRAK4 kinase activity, IRAK1 phosphorylation, NF-κB signaling, JAK kinase activation of STAT3, and secretion of interleukin (IL)-6, IL-10 and interferon-β [22]. Recently, the same *MYD88* L265P mutation has been found to be present in 49 out of 54 patients with Waldenstöm's macroglobulinemia and in 3 of 3 patients with non-IgM-secreting lymphoplasmacytic lymphoma [23]. However, whereas the clinical manifestation of DLBCL is mostly dependent on uncontrolled tumor growth, the clinical manifestation of Waldenstöm's macroglobulinemia is also associated with dysfunctional B cells, which cause paraprotein-related cryoglobulinemia, cold agglutinin syndrome, and demyelinating neuropathy [23]. It is unclear to what extent the *MYD88* L265P mutation contributes to the distinct aspects of pathogenesis in these two diseases. It is conceivable that the same somatic mutation has very different effects, depending on the cell type of origin in which it occurs. This may lead to different degrees of uncontrolled cell growth as well as different types of cellular malfunction in these two types of lymphoma.

While the same *MYD88* mutation may occur in very distinct lymphoid malignancies, *MYD88* is also critically involved in immune tolerance. *FOXP3*-deficient mice have a defect in the generation of regulatory T cells that suppress self-reactive T cells and contain immune responses [3], and this deficiency mimics the human IPEX syndrome. The autoimmune syndrome that develops in these mice is ameliorated on a *MYD88*-deficient background, suggesting that tonic MYD88 signaling is involved in the manifestation of this autoimmune disease [24].

Chronic B cell receptor stimulation by self-antigens leads to poorly competitive B cells, which undergo rapid cell death, as a mechanism of tolerance [25]. CD79B is part of the B cell receptor complex, and significantly mutated in DLBCL [18,26]. Instead of functioning as an oncogene that leads to cell autonomous proliferation, *CD79B* mutations were proposed to allow the B lymphocytes to respond abnormally well to a self or foreign antigen [26]. This mutation may therefore promote an initial selective advantage of the affected B cells and may alter B cell responses to antigen, but it is insufficient as an oncogene that causes autonomous tumor growth. These examples highlight two points. First, similar pathways may be activated in lymphoid neoplasms and autoimmune diseases, and thus, therapeutic targeting of these pathways may be advantageous in both disease groups. Second, similar genetic defects may be present in clinically very distinct diseases. The pathogenic effect of a specific mutation may depend on the cell of origin in which it occurs, the state of differentiation and the microenvironmental context.

A wealth of genomic data on lymphoid neoplasms has been generated over the last 4 years by deep sequencing, including DLBCL, Burkitt lymphoma, multiple myeloma, and chronic lymphocytic leukemia (CLL) [18–21,27–29]. The functions of many of the mutated genes are unknown. More work is needed to unravel the signaling pathways, which are activated by these mutations, and to determine if these pathways are activated in autoimmune diseases as well.

3. Clonal evolution in autoimmunity and lymphoid malignancies

Immune responses are characterized by expansion and contraction of clones, changes in function over time, and heterogeneity with regard to their phenotype. We developed a model in which antigen specific T cells (DO11) were adoptively transferred into mice that ubiquitously express the cognate antigen as self, i.e. in the absence of any inflammatory stimuli, as a model for autoimmunity. All T cells were only able to express only one type of T cell receptor, as they were crossed onto a *RAG1*−/− or *RAG2*−/− background. Upon transfer into a lymphopenic animal expressing the cognate "self" antigen, the T cells underwent a massive expansion without subsequent contraction (Fig. 1) [30,31]. The functional phenotype of these cells followed very reproducible kinetics: The initial phenotype was characterized by differentiation into Th17 cells with secretion of IL-17. Over time, the Th17 cells disappeared, and Th1 cells, as defined by the secretion of IFNγ, became dominant [31]. These first two phases were associated with a massive clinical phenotype that displayed various signs of systemic autoimmunity and also graft-vs-host disease. Eventually, a population of FOXP3 positive regulatory T cells emerged leading to the clinical recovery of the animals with re-establishment of tolerance. In contrast, if the same T cells were exposed to the same antigen in a mouse that was not lymphodeficient, no clinical phenotype was observed. The T cells underwent a brief stimulation with modest expansion and IL-2 production, which was quickly followed by deletion of these cells, and anergy induction of the few remaining cells [32]. The remarkable plasticity of lymphocytes in this case is particularly striking, as the antigen is constant for all lymphocytes, and the lymphocytes are essentially clonal. In other words, different paths of differentiation result in the emergence of different subclones over time, despite being derived from the same "monoclonal" population, and reacting to the same stimulating antigen. Similar observations were made for B lymphocytes [33], and much work has been done to elucidate the molecular mechanisms, which direct these cells towards one or another differentiation pathway. Several parameters may impact the differentiation

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