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Shared and unique immune alterations in pre-clinical autoimmunity

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Progression from health to a classified autoimmune disease is an evolving process that can happen rapidly in some diseases, but usually takes years to develop. Specific immune alterations predate pathogenic autoimmunity and can be used as disease biomarkers to identify high-risk individuals for prevention studies applied in the pre-clinical state. Here we discuss recent findings that illuminate specific immune pathways that are altered in the earliest phases of pre-clinical autoimmunity as well as those mediators more closely associated with later clinically apparent and classified disease onset.

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Introduction

Autoimmune disease develops from aberrant immune responses against self-proteins and molecules, along with other associated immune dysregulation, in a process which damages tissues and leads to classified disease. Loss of self-tolerance, typically detected by the appearance of autoantibodies, occurs years before disease onset when individuals appear physically healthy and without organ damage. This period of pre-clinical autoimmunity allows study of pathogenic mechanisms that initiate disease and introduction of primary and secondary preventative strategies. This review discusses recent advances in understanding altered immune responses in this pre-clinical phase using RA, SLE and T1D as examples.

Autoantibody seroconversion, specificities, and progression to clinical disease

Autoantibodies are a hallmark of autoimmune diseases. Although autoantibody positivity does not necessitate transition to clinical autoimmune disease, disease-associated autoantibodies can be found in some individuals more than 10 years before disease classification [1,2••,3–5]. In SLE, patients accumulate an average of three autoantibody specificities before diagnosis [2••,6]. The earliest autoantibody detected in pre-clinical subjects before SLE classification is anti-Ro, followed by autoantibodies to RNP, chromatin, Sm, dsDNA, and La [2••]. In unaffected blood relatives of SLE patients, those who later transitioned to SLE were more likely to have anti-Ro/SSA and anti-RNP and had more lupus-specific autoantibody specificities at baseline (~6 years before SLE diagnosis), compared to relatives who did not transition to SLE [7]. However, neither anti-nuclear autoantibody positivity nor number of specificities alone could strongly predict future SLE transition in unaffected relatives of SLE patients [7]. Other studies have identified autoantibodies that suggest the absence of a systemic autoimmune disease. For example, anti-dfs70 is more prevalent in healthy individuals, and autoimmune disease patients rarely have anti-dfs70 as a predominant or isolated anti-nuclear specificity [8,9]. Therefore, autoantibody profiles may be useful for detecting increased risk, excluding autoimmune diagnoses, or discerning a lower risk for future clinically apparent disease.

In RA, levels of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) increase a median of 7.4 years before onset of RA and the ACPA repertoire spreads closer to disease onset, with increases in IgG and IgA reactivity to cyclic citrullinated peptide-2, cyclic citrullinated peptide-3, citrullinated α-enolase peptide, fibrinogen β36-52, and filaggrin levels [4,5,10–12]. Anti-carbamylated protein (anti-CarP) antibodies were found to demonstrate high specificity, but low sensitivity, for future RA [13]. Anti-CarP antibodies neither improve diagnostic accuracy when added to ACPA and RF, nor antedate the development of ACPA and RF [13]. Furthermore, ACPAs cross-react with carbamylated proteins, and few ACPA-RA patients are anti-CarP+ (~4%)—, suggesting some anti-CarP antibodies may in part represent a cross-reactive subset of ACPA [14]. Thus, ACPA and RF still remain the most informative autoantibody markers for the development of future seropositive RA. However, over one-third of RA patients are seronegative in early disease, underscoring the need for other predictive analytes in that population [15].

The progression of pre-clinical T1D corresponds to the accumulation of autoantibodies against pancreatic β cells, most commonly glutamic acid decarboxylase (GAD-65), insulin (IAA), insulinoma-associated antigen 2 (IA2A), and zinc transporter 8 (ZnTA8). Individuals with reactivity to only a single islet antigen have a lower risk of clinical progression, whereas individuals with multiple islet autoantibodies have a 70% chance of developing disease within 10 years [16,17]. In addition, the rate of progression to clinical disease varies with specific autoantibodies. IAA is the most common initial autoantibody specificity in childhood, followed by GAD-65; however, IAA or ZnTA8 seroconversion occurred at an earlier age versus GAD-65 or IA2A seroconversion. Individuals who remained diabetes-free for over 10 years with multiple autoantibodies were older and more likely to have low levels of IAA [18,19]. Further, children presenting with anti-IA2A progressed rapidly and those with anti-ZnTA8 progressed slowly [18,20].

In high-risk blood relatives of type 1 diabetics, treatment with a single course of teplizumab (anti-CD3) delayed the time to diagnosis of type 1 diabetes, with annualized rates of diagnosis of 14.9% per year in the teplizumab group and 35.9% per year in the placebo group [21••]. Participants with anti-ZnTA8 had a reduced response to teplizumab (hazard ratio 0.07, 95% CI, 0.02–0.26), while the response to treatment was not affected by anti-GAD65. Minor, non-islet associated autoantibodies have also been found during the pre-clinical phase of T1D. Nuclear and mitochondrial targeted autoantibodies such as NUP50, MLH1, PPIL2 and MTIF can be found at T1D diagnosis and may contribute to disease susceptibility [22]. Autoantibodies remain a critical immune alteration found during the pre-clinical phase of autoimmunity where particular antibody specificities, rising concentrations, and epitope spreading precede and often associate with disease transition.

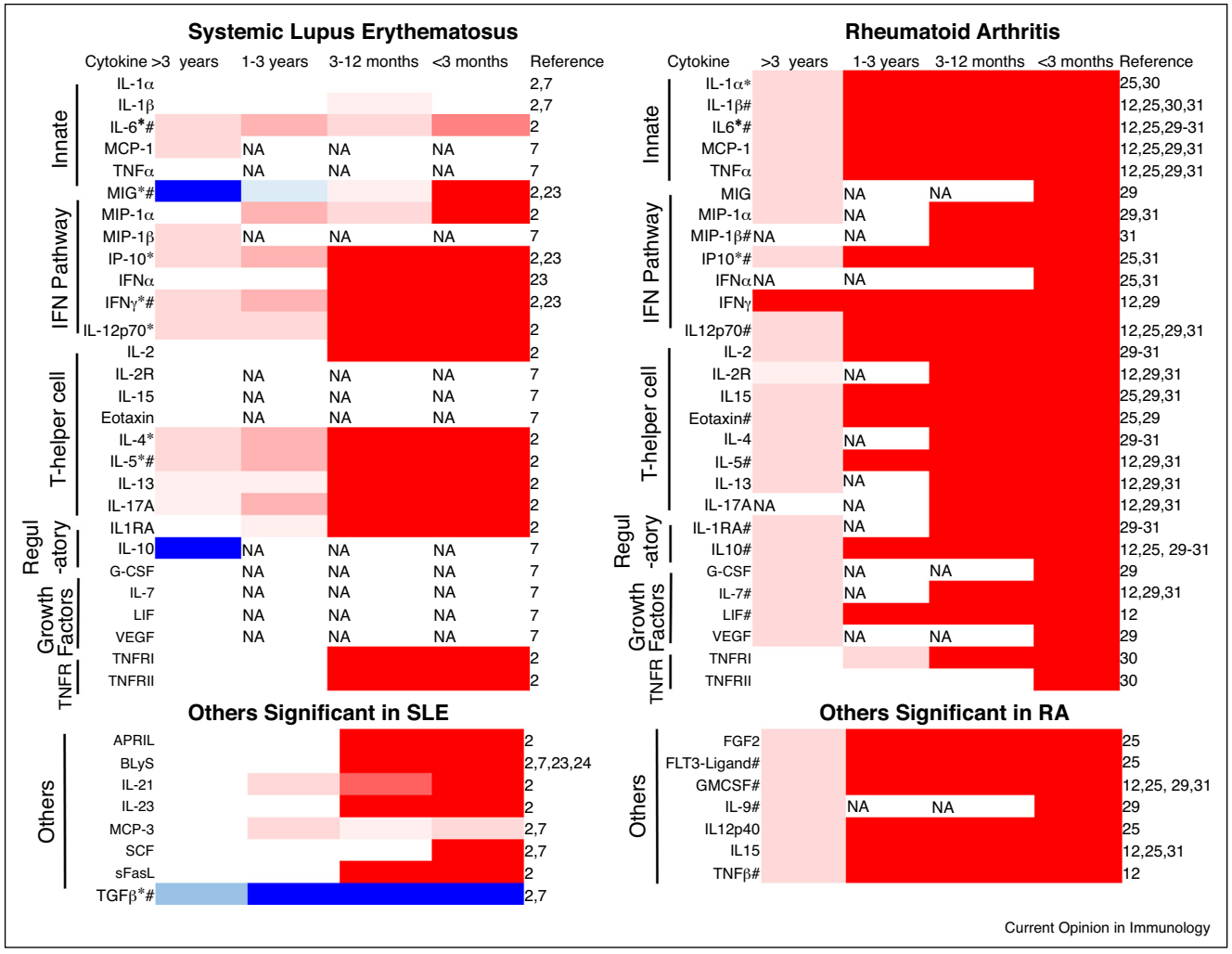
The rise of soluble mediators in pre-clinical autoimmunity

Autoantibodies are not the only early indication of an autoimmune disorder. Circulating cytokines can increase concurrent or before autoantibody production [2••,23•,24,25]. Type I IFNs are proposed to play a central role in lupus pathogenesis, and IFN-inducible gene expression and IFN-associated cytokines precede disease onset [2••,7,23•,26–28]. Type I IFN and associated mediators become elevated after autoantibody seroconversion and near the time of SLE classification, similar to BLyS, APRIL, SCF, TNFR1, and TNFR2 [2••,23•,24]. Other mediators are elevated earlier in the pre-clinical period, either preceding or coinciding with autoantibody positivity; type II IFN, type II IFN-associated mediators (IP-10, IL-12p70, and MIG), IL-4, IL-5, and IL-6 are elevated >3.5 years before SLE classification [2••,23•] (Figure 1). Indeed, IFNγ is one of the first reported cytokines to appear in lupus patients, further emphasizing the importance of Type II IFNs in early autoimmunity [23•]. In healthy SLE relatives who later transitioned to clinical disease, regulatory TGFβ and IL-10 decreased from baseline to disease transition [2••,23•]. Together, these observations suggest that early imbalances in critical regulatory pathways, type II IFN, and T cell-related pathways are associated with and may lead to an initial loss of tolerance in SLE [23•], followed by increasing disruption of immune homeostasis that precipitates clinical disease [7].

In RA, multiple cytokines are tied to disease pathogenesis, including IL-1, TNFα, and IL-6, which are the targets for currently licensed drug treatments in RA. These cytokines, along with others (IL-10, IL-15, IL-12p40, GMCSF and IP-10) can be detected up to 7.2 years before clinical diagnosis of seropositive RA, and higher cytokine concentrations are associated with more rapid progression [12,25,29,30] (Figure 1). In a subset of subjects, serum levels of several cytokines, IL-1α, IL-6 and IP-10 rise before anti-CCP seroconversion [25]. Furthermore, certain cytokines are elevated in RA patients, arthralgia patients, and unaffected first-degree relatives, and differentiate them from healthy controls regardless of autoantibody positivity [31•]. Seronegative RA patients were distinguished by elevated serum levels of the regulatory cytokine IL-10, and reduced levels of eotaxin and RANTES compared to healthy controls [31•]. High risk seropositive arthralgia patients tended to demonstrate increasing levels of IL-5, MIP-1β, IL-1RA, and IL-12 before RA development compared to non-progressors, suggesting dysregulation in multiple T-helper (Th)1, Th2 and monocyte driven pathways leading to RA development [31•]. Of interest, IFNα only became significantly elevated in serum after RA diagnosis [31•,32].

The involvement of soluble mediators in T1D pathogenesis is suggested by elevated levels of IL-17, TNFα, and IL-6 in patients [33–35]; however, little is known regarding soluble mediator levels before T1D onset. Similarities in altered soluble mediator pathways exist before disease development among autoimmune diseases, with elevated Type II IFN, Th2, and IL-6 pathways being elevated >3 years before disease development, and Type I IFN arising just before clinical onset (Figure 1). It would be beneficial to evaluate soluble mediators across autoimmune diseases using the same experimental technique to establish commonalities and disease-specific pathways. The usefulness of systemic cytokines in deciphering altered immune pathways before autoimmune disease is only beginning to be unraveled; however, these mediators may improve the predictive accuracy for disease transition, especially during the seronegative time period in high-risk individuals.

Figure 1

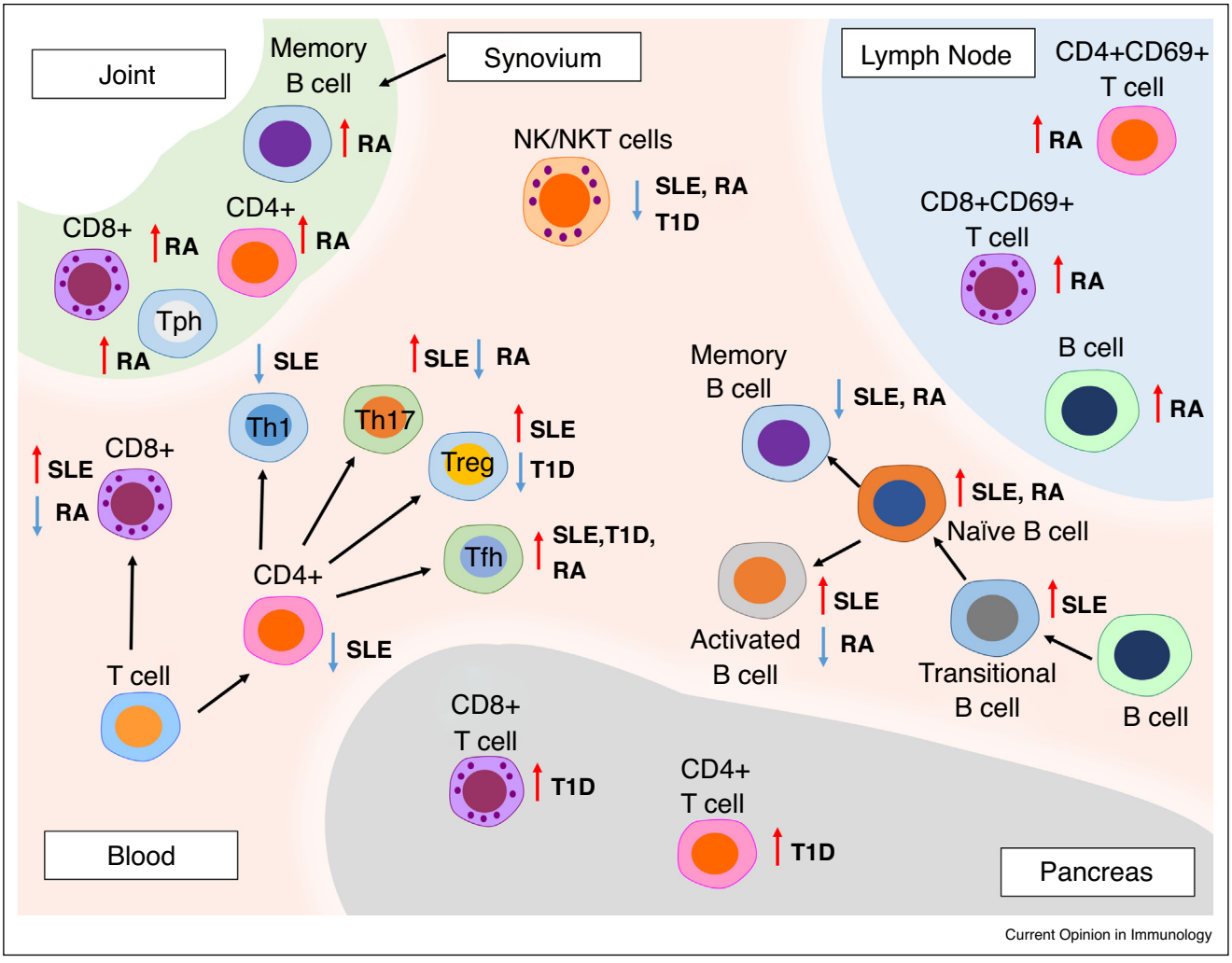


Cytokines are dysregulated years before RA and SLE development.
Relative levels of dysregulated cytokines in the periphery are shown according to time of appearance for subjects at-risk and/or progressing to autoimmune disease development compared to healthy controls. Cytokines with no significant change compared to healthy controls are shown in white. Cytokine levels that are increased before disease development compared to healthy controls are shown in red, while cytokines that are decreased are shown in blue. Darker colors indicate a greater difference between cases and controls. Cytokines with no available information for a particular time point are indicated by NA. Cytokines that appear before autoantibody development are marked with an asterisk (*). Those soluble mediators used in predictive models are marked with a numeric symbol (#). Data sets compiled and used to generate relative levels in each row are noted in the far right of each table within the reference column. As plasma/serum cytokines were run in different studies under various conditions, it is important to note that relative levels are not directly comparable between diseases, nor potentially between studies.

Systemic changes in immune cell frequencies and phenotype before disease

Alterations in immune cell frequencies and activation have been extensively studied in patients with established autoimmune diseases, often with conflicting results due to variability in disease stage, medication use, clinical symptoms, disease activity, and analytic approach. The pre-clinical phase provides a unique window to view immune cell abnormalities that may involve pathogenic disease pathways. In autoimmune rheumatic diseases, reductions in neutrophils and lymphocytes are common [36]. Of interest, both ANA+ healthy individuals and patients with undifferentiated connective tissue disease (UCTD) exhibit reductions in total peripheral blood mononuclear cells, primarily attributed to decreased CD4+ T cell and NK cell numbers, compared to ANA− healthy controls [37,38]. ANA+ healthy individuals and UCTD patients also exhibit alterations in B cells, including increased naïve B cells and CD86 expression in UCTD patients and elevated relative frequencies of memory B cells and plasmablasts in ANA+ healthy individuals [37,38] (Figure 2).

Figure 2



Model of cell frequency differences of lymphocytes at the time of clinical autoimmune diagnosis.
At the time of SLE diagnosis, an increase in activated B cells can be seen in the periphery, while decreases in NK cell and CD4+ T cells are observed in the blood. In RA, an influx of CD8+ and CD4+ T cells (particularly PD-1^{hi} CXCR5-Tph cells), along with memory B cells is observed in the synovium, with matched decreases in these cell populations in the periphery. Further, increased numbers of activated T cells and B cells are also observed within the lymph node of recently diagnosed RA patients. T1D onset is also associated with infiltration of the pancreas with CD8+ and CD4+ T cells that are localized outside of the islets. Further, decreases in NK cells are also observed in the periphery with T1D onset.

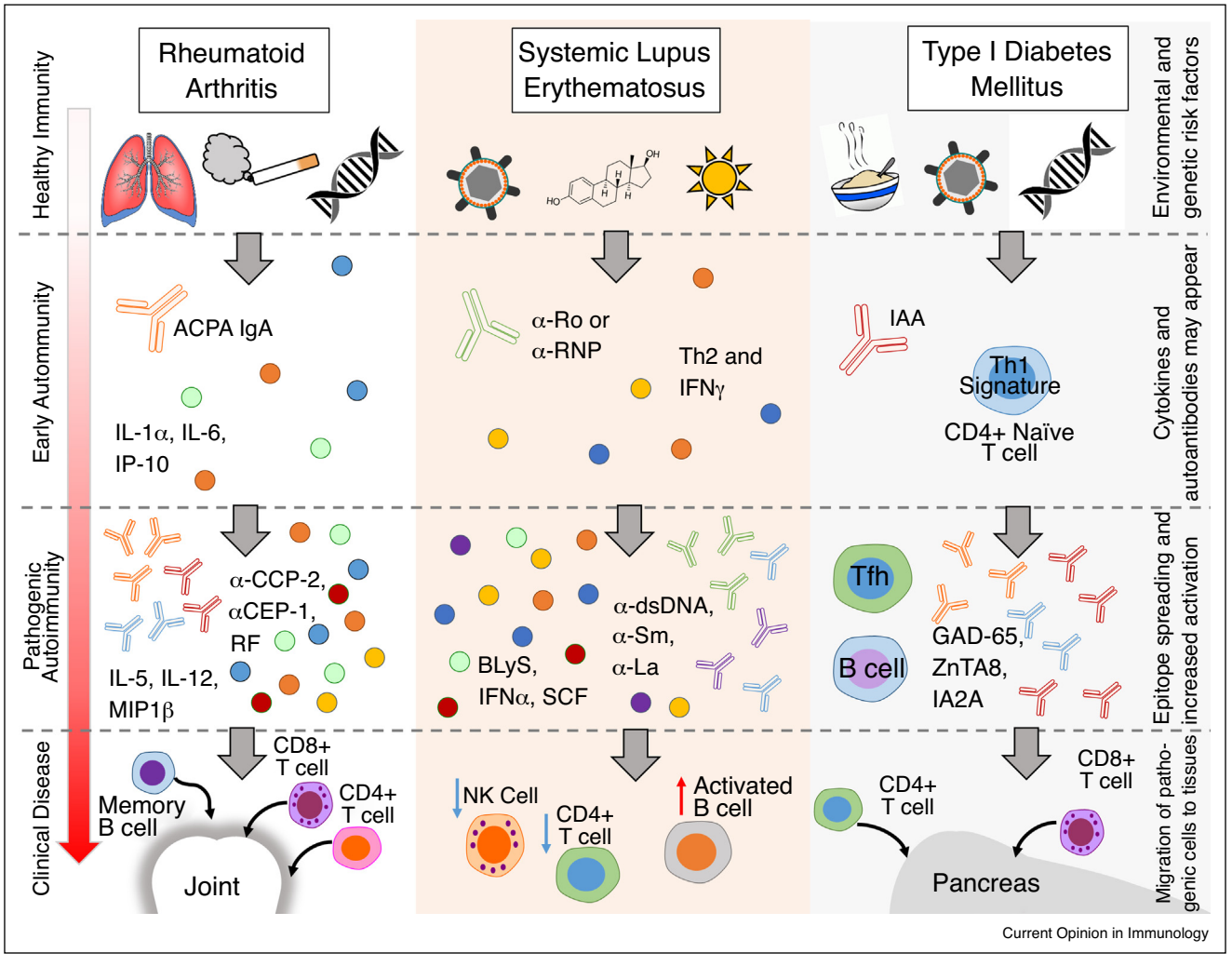
The expansion of effector T cells in patients with active RA is associated with tissue-specific destruction; namely, Th22, Th17, Tfh (T follicular helper) and Tph (peripheral T helper-PD-1^{hi}CXCR5−) CD4+ T cells are implicated in RA pathogenesis [39,40]. In addition, recent studies suggest that alterations in circulating CD4+ and CD8+ T cell compartments precede RA onset [41•,42•]. Of interest, early RA patients have elevated Th1 cells [43], and preclinical RA involves an imbalance of effector T cell subsets with regulatory T cells (Tregs) along with dysfunctional inhibitory pathways, such as PD-1 [43–46]. B cells are also altered in pre-clinical RA. Arthralgia patients who progress to RA have reduced frequencies of peripheral memory B cells 12 months before diagnosis and relative decreases in CD80+ B cells near the time of diagnosis, compared to non-progressors [41•]. When examining plasmablasts in subjects at risk for future RA, an elevated proportion of highly mutated IgA plasmablasts was found [47]. In addition, clinical onset of RA was associated with higher numbers of dominant B cell receptor (BCR) clones in the periphery, with activated and dominant clones appearing in the synovium near the time of clinical onset [41•,48•]. Confirming the importance of B cells in early RA pathogenesis, a single 1000 mg infusion of rituximab decreased the risk of RA development by 55% after 12 months of follow-up in RF+ and ACPA+ healthy subjects at high risk for RA disease transition [49•].

In T1D, both CD8+ and CD4+ effector T cells are recruited to β cell islets just before T1D onset [44,50•]. T follicular helper (Tfh) cells are of particular interest due to their B cell helping capabilities and early autoantibody production; indeed, elevated frequencies have been observed in ANA+ healthy individuals and autoantibody-positive at-risk T1D children, and increase just before presentation of T1D [38,51]. In a landmark longitudinal study, β cell autoantigen-specific naïve CD4 + T cells of children who develop β cell autoimmunity expressed a unique signature of pre-Th1/Th17/Tfh cells in infancy, before appearance of autoantibodies or T1D specific memory T cells [52••]. This signature shifted to an IFNγ-Th1 memory phenotype at the time of autoantibody appearance, strengthening the importance of Type II IFN pathways across autoimmune disease initiation [2••,23•,52••]. Similar to RA, dysregulation in the PD-1 regulatory pathway was associated with T1D onset, confirming the importance of inhibitory receptors in autoimmune progression [53]. More integrated approaches highlighted alterations in immune cell frequency and activation phenotype with cytokine and autoantibody production are essential in understanding the earliest immune events leading to pathogenic autoimmunity.

Disturbances in signaling pathways in pre-clinical autoimmunity

Gene expression profiles have helped elucidate active pathways important in the progression from pre-clinical autoimmunity to clinical autoimmune disease. For instance, IFN pathways are dysregulated in a significant proportion of SLE, RA, and T1D patients, and these

Figure 3



Summary of early immune differences that arise in RA, SLE, and T1D.
The path to disease progression likely begins with genetic and environmental factors that predispose certain individuals to the disease. Early autoimmunity is recognized by the appearance of autoantibodies and elevated pro-inflammatory cytokine levels. Early autoimmunity can proceed to pathogenic autoimmunity with epitope spreading and expansion of autoantibodies, increasing cytokine levels, and cell activation. Clinical disease development is characterized by the migration of activated cells within the tissues and symptom onset.

findings can be extended to the pre-clinical period [27,32,37,54•,55–57,58•,59]. ANA+ healthy monocytes and B cells exhibited altered phospho-STAT signaling in response to IFNα, and a subset (36.8%) had elevated IFN inducible gene expression [27,37]. Further, ACPA+ individuals at high risk of RA had upregulated type I and type II IFN-inducible genes, and like early RA patients, showed upregulation in the functional pathways of WNT signaling, TCR signaling, regulation of cytokine synthesis, clotting, and the complement system [54•]. The primary contributors of the whole blood IFN signature in pre-clinical RA and T1D was neutrophils, which were decreased systemically at the time of diagnosis and localized in disease affected tissues [59,60•]. Further validating the significance of IFN inducible genes in autoimmune disease progression, dysregulation of IFN signature genes associated with Th2 regulation and B cell differentiation correlated with ACPA and anti-CarP antibody positivity in ACPA+ healthy and RA subjects [32]. However, use of the IFN signature as a potential biomarker may be limited by the heterogeneity in the signature, and the absence of elevated IFN in some patients pre-clinically or after diagnosis. Thus, the need for understanding other altered pathways is warranted [61]. Other pathways linked to early autoimmune dysregulation occur due to impaired induction, such as PTPN22 and T cell exhaustion markers, in subjects at high-risk of RA [42•,46]. The elucidation of these specific pathways and timing of pathway dysregulation will be important in determining the events leading to pathogenic autoimmunity.

Pre-clinical studies across autoimmune diseases

Studying early autoimmunity across diseases is critical in the move toward prevention and improved disease understanding. Similarities exist between dysfunctional immune pathways across SLE, RA and T1D. Moreover, disease-associated autoantibodies were recently found to cross disease-specific boundaries in SLE, RA and T1D, and also within SLE and RA patient first-degree relatives [62••]. This alternative autoimmunity (the presence of at least one autoantibody not associated with the disease of interest) or expanded autoimmunity (the presence of antibodies associated with two alternative autoimmune diseases) was more common with SLE, and was present at high frequencies in SLE and RA first-degree relatives [62••]. The appearance of alternative autoimmunity before disease development and similarities to autoantibody patterns in clinical disease suggest that dysfunctional immune patterns are established and carried from the pre-clinical to the clinically apparent and classified autoimmune disease phase. Our understanding of altered immune pathways in the pre-clinical phase of autoimmunity will greatly increase as we assess and compare phenotypes across multiple diseases.

Conclusions

Immune alterations in the pre-clinical phase of autoimmunity provide fundamental biomarkers that are important for early targeting and understanding of critical pathways leading to pathogenesis. Recently, some of these biomarkers have been used to identify high risk subjects in some of the first ever prevention trials (DPT-1 [NCT00004984], Stop-RA [NCT02603146], and SMILE [NCT03030118]) for subjects in the pre-clinical autoimmune phase. Combinations of autoantibodies and cytokines may further improve our predictive ability for autoimmune disease development, both with regard to the positive predictive value for future disease and a shorter median time to clinically apparent and classified disease onset. As our understanding of the pathways, cell populations and soluble mediators (summarized in Figure 3) that contribute to progression and pathogenesis improve, earlier identification and the likelihood of disease prevention are increasingly coming within reach.

Conflict of interest statement

Nothing declared.

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