Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is one of the most common chronic inflammatory diseases and in modern times has become a prototype disease entity for defining the molecular and pathological basis of chronic inflammatory syndromes. The term *rheumatoid arthritis* was coined by Garrod in 1859. However, this was probably an inappropriate use of the term because it encompassed polyarticular osteoarthritis as well as inflammatory polyarthritis. In spite of references to inflammatory afflictions of joints by the likes of Galen, Sydenham, and Heberden, the first convincing case reports of the disease, described in terms that would be recognizable today, were published in 1800 by Landré-Beauvais, who labeled the disease "la goutte asthénique primitive." This description was distinct because all patients were female, an observation that was significant when the most important differential diagnosis at that time was polyarticular gout, a disease predominantly of males.

Today we recognize RA as a chronic inflammatory disorder of joints of unknown etiology in which the major target tissue is the synovial lining of joints, bursae, and tendon sheaths. Although traditionally considered an autoimmune disease, RA differs from organ-specific autoimmune disease entities in several respects.[1] From the outset of clinically apparent disease, the systemic immuno-inflammatory process, driven by cytokines and other inflammatory mediators, promotes the activation and proliferation of stromal joint tissues, in particular the fibroblastic synovial lining layer. This appears to contrast with organ-specific autoimmune diseases, such as type 1 diabetes or autoimmune thyroiditis, characterized by an antigen-driven immune-inflammatory response *in situ* leading to targeted cellular destruction of autoantigen-expressing pancreatic β-islet or thyroid tissue cells. In RA, once the inflammatory process is established, the inflammatory synovium, or pannus, may invade and erode underlying cartilage and bone. Unlike autoimmune diseases that target single organs or tissues, RA is a systemic inflammatory disease that likely encompasses a heterogeneous syndrome with marked variation in clinical expression that most clinicians today would acknowledge is more than one disease entity. Indeed, it is now apparent that the disease is heterogeneous not only clinically but also pathologically, serologically, and genetically.

EPIDEMIOLOGY

The incidence of RA (the rate of new cases arising in a given period) is approximately 0.4 per 1000 when the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria are applied. Large cross-sectional population samples indicate that disease prevalence, which ideally should include all past and inactive cases, ranges from 0.5% to 2% for Caucasian European and North American populations over the age of 15, with a female to male excess of 2 to 4 times. Rates plateau between the ages of 45 and 75 years in some series, but can increase steadily with age until the seventh decade, declining thereafter. Despite similar prevalence estimates for these geographically diverse populations, greater diversity has been documented for rural African populations, where the prevalence has been reported to be as low as 0.1%, and for Native Americans (including the Pima, Yakima, and Chippewa tribes), where the prevalence may be as high as 5%. Such variance across geographical borders likely reflects distinct environmental factors and sociodemographic determinants, as well as a spectrum of genetic admixture. Lifetime risk has been estimated at 2% for males and 4% for females.

Complex polygenic autoimmune syndromes like RA are diseases of low penetrance, where thresholds of disease expression may be higher in males. Recent insights into familial clustering indicate that family history remains a strong independent risk factor for RA, but that this risk does not differ by gender.[2] This implies that nongenetic factors influence gender bias. Twin studies also provide compelling evidence for genetic effects, given the excess concordance rates for monozygotic (12% to 15%) compared with dizygotic twins (<5%, and probably nearer to 3.5%).[2] These concordance rates appear somewhat low, but compared with a background prevalence of 1% in outbred populations, genetic epidemiology studies report heritability estimates of 68% for those patients who carry antibodies to citrullinated protein antigens ($ACPAs^+$) and 66% for those who do not ($ACPAs^-$), indicating substantial genetic influence.[3] Human leukocyte antigen (HLA) is thought to contribute 35% to 40% of this value. This "missing heritability" leaves a substantial contribution to disease susceptibility from environmental factors, influenced by occupation, socioeconomic status, exposure to infectious pathogens, and lifestyle factors—a conglomeration of factors termed the "exposome."

Two of the more intriguing factors contributing to disease occurrence are age and gender. Age-associated changes in susceptibility to infection, neoplastic disease, and autoimmunity suggest that a common mechanism could be responsible. Immune senescence is one possibility, where age-related decline in host immunity is characterized at the cellular and molecular level by expansions of lymphocyte clones, corresponding contractions of the naïve T- and B-cell repertoires linked to depletion of lymphocyte precursors in thymus and bone marrow, and telomere erosion of leukocytes, features indicative of an extensive proliferative history. When combined with dysregulation of costimulatory receptors such as CD28, oxidative stress,

KEY CONCEPTS

Important Risk Factors for Developing Rheumatoid Arthritis

- Female gender; impact of X chromosome, micro-chimerism, lifestyle
- Age; associated with accelerated immune aging
- Inheritance of genetic variants, *e.g., HLADRB1* and *PTPN22*
- Autoantibodies to modified protein antigens (AMPAs), rheumatoid factor
- Family history; first-degree relatives have higher prevalence of genetic and serological risk factors
- Hormonal factors; nulliparity, the first 3 months postpartum, low androgen or high estrogen status (in males); longer-duration breastfeeding
- Smoking status; >25 cigarettes/day for >20 years confers a 15-fold risk in subjects who carry disease associated human leukocyte antigen (HLA)-*DRB1* alleles
- Low alcohol intake
- Environmental antigens (the "exposome"); dietary factors; exposure to infectious (and noninfectious/microbiota) pathogens at mucosal surfaces such as the lung, periodontium, and gut; non-inherited maternal antigens (NIMA)
- Urban dwelling, relating to airborne pollutants

and a range of biochemical derangements of pathways integral to antigen responsiveness and immune regulation, these factors may combine to (1) increase susceptibility to foreign pathogens, (2) augment reactivity to self-tissue antigens (which may be modified post-translationally by the aging process), and (3) generate a repertoire of lymphocytes defective in terms of tumor surveillance. Thus, physiological immune senescence could be considered a risk factor for RA in the elderly, while premature senescence may contribute to early onset RA.

The female sex preponderance implies that hormonal and reproductive factors strongly influence risk. On the one hand, nulliparity is a risk factor for RA. Women entering the first 3 months of the postpartum period are also at increased risk. By contrast, oral contraceptive use, pregnancy, and hormonal replacement therapy have all been associated with reduced risk or less severe disease, whereas extended periods of breastfeeding appear to increase risk. An influence of hormonal factors is further suggested in studies of men where disease is associated with lower androgenic testosterone and dehydroepiandrosterone (DHEA) levels, and increased estradiol, compared with healthy control male subjects.

ETIOLOGY AND PATHOGENESIS

Environmental and Nongenetic Factors

Most, but not all studies have reported an association between RA and smoking. One of the largest studies, comprising over 370,000 women from Women's Health Cohort Study, reported a relative risk of 1.4 for women who smoked more than 25 cigarettes per day for more than 20 years, compared with nonsmokers.[4] The association appears to be more closely related to duration than to the amount of tobacco exposure, with smoking status being a risk factor for older age of RA onset; smoking also may influence severity because smokers are more likely to have seropositive, erosive disease with extraarticular manifestations. Further evidence of gene-environment interactions with respect to smoking has been documented in a population-based case–control study of Swedish RA patients.[5] In this study the relative risk of developing rheumatoid factor (RF) positive (RF^+) RA was calculated according to smoking status and HLA-DRB1 genotype. The relative risk of developing RA increased from 2.5 in nonsmokers with disease-associated HLA-DRB1 genes to 7.5 and 15.7 in smokers who carried one or two copies of the susceptibility alleles, respectively. Follow-up gene-environment interaction studies demonstrate robust associations between heavy smoking, HLA-DRB1 alleles encoding specific amino acids at positions 11 and 13, and the presence of antibodies to citrullinated protein antigens (ACPA). Strongest associations were with antibodies to citrullinated α and β chains of fibrinogen (epitopes Fibα580–600 and Fibβ36–52) and α-enolase (CEP-1); IgA ACPAs were especially prevalent in smokers.

Being female, a smoker, and carrying specific disease-associated genetic variants may be necessary but not sufficient to initiate chronic inflammatory arthritis. Other environmental triggers may be involved. Not least among these is exposure to foreign pathogens. This association has gained credibility because of the presumed link not only between infection and autoimmunity but also between immunodeficiency and autoimmune disease. Nonetheless, no single pathogen or group of pathogens has been defined. This could imply that aberrant host responses (either exaggerated innate inflammatory responses or failure to terminate such responses) may arise after a wide range of infectious insults. Indeed, bacterial products including superantigens, mycoplasma species, viruses (including herpes family, parvovirus, and retroviruses), and fungi have all been implicated, but data are insufficient to prove causation. Epstein-Barr virus (EBV) infection is common, and antibodies to EBV nuclear antigens have been reported in patients with RA. EBV is a polyclonal activator of B lymphocytes and EBV-specific T cells reactive to EBV gp110 have been identified in RA synovial joints, in keeping with the detection of EBV RNA in synovium. There exists a tantalizing link between infection with *Porphyromonas gingivalis*, which expresses its own enzymatic machinery for generating bacterial or host-derived citrullinated proteins, severe periodontitis (which shares risk factors for RA), and RA.[6]

A comparison of the microbial genomes from the small intestine and colon of mice housed in conventional versus germ-free facilities suggests that a single gut-residing species, in this case segmented filamentous bacteria, can profoundly influence the clinical expression of inflammatory arthritis in mouse models of autoimmune arthritis. In the K/B × N serum transfer model of arthritis, segmented filamentous bacteria enhanced generation of interleukin (IL)-17-expressing T cells in lamina propria. Together with rapid advances in sequencing technologies, these data have prompted a systematic analysis of the symbiont microbial communities in patients with RA in comparison to those derived from healthy control populations. Microbiota are attractive environmental risk factors because they are acquired at around the time of birth and are modified by diet as well as the host genome. Studies from several groups have demonstrated irrefutable evidence of dysbiosis, with distinct patterns of microbiota depending on the stage of disease and the population studied. For example, the first US study reported enrichment of *Prevotella* spp. and *Bacteroides* spp., gram-negative anaerobes in the gut of patients with early but not established RA. Metagenomic sequencing of oral and fecal microbiota from a cohort of Chinese RA patients revealed enrichment of *Lactobacillus*, particularly in severe disease, and depletion of *Haemophilus* spp.[7] Recent studies indicate that enrichment of *Prevotella* spp. is associated with an RA polygenic risk score in unaffected twins, as well as subjects at risk of RA,[8] suggesting a link between host genetic factors and dysbiosis prior to disease onset.

Immunogenetics

RA is a clinically heterogeneous disease, and so comprehensive identification of disease susceptibility genes has been challenging, in spite of heritability estimates in excess of 60%. With the exception of the MHC, where extensive gene polymorphism contributes about one-third of genetic susceptibility, and *PTPN22* (odds ratio 1.8), individual genetic variants confer low to moderate risk and have low penetrance (odds ratios of 1.1 to 1.5). Numerous genome-wide linkage scans of multiplex families with RA have established and confirmed an important contribution of the MHC (Chapter 5). This lends support to a wealth of epidemiological and genetic data describing associations between RA and specific HLA-*DRB1* alleles, in particular HLA-DR4 subtypes. Although this association was first described by Stastny in the 1970s, it was shown more than a decade later that susceptibility to RA across different ethnic populations correlated closely with the expression of a specific consensus amino acid sequence (referred to as the "shared epitope," hereafter SE) within the HLA-DR β chain α-helix (Fig. 53.1).[9] This sequence was subsequently shown by several groups of investigators to be encoded by HLA-*DRB1* alleles, including *HLA-DR4 (*04:01, *04:04, *04:05*, and **04:08)*, but also *HLA-DR1 (*01:01), DR6 (*14:02)*, and *DR10 (*10:01)* alleles, among others. *HLA-DR9 (*09)* is also associated, but not in Caucasians. According to fine-mapping data, RA risk is linked not only to amino acids encoded by the RA SE sequence (71 and 74 positions of the alpha helix of the DRβ chain) but also to amino acid positions 11 and 13, located in the peptide binding groove of the HLA-DR heterodimer.[10] Single amino acid variants have also been defined in HLA-B (position 9) and HLA-DPβ (position 9). Differences in odds ratios associated with these five amino acid variants between ACPA-positive and ACPA-negative disease provide further evidence that seropositive and seronegative disease are genetically distinct.

Specific genotypes co-segregate with distinct clinical features. For example, in population-based studies, different HLA-*DRB1* alleles influence the severity of disease, including radiographic progression (confirmed in meta-analyses), with *DRB1*0401* being found in patients with severe, seropositive, erosive RA (often with extraarticular features such as vasculitis and Felty syndrome in *04:01 homozygous or *04:01/*04:04 compound homozygote individuals). Valine at position 11 of HLA-*DRB1* confers the strongest association with radiological damage and highest all-cause mortality. Statistical modeling points to HLA genetic polymorphism being associated with young-onset RA. *DRB1**01:01 and *10:01 are observed at a higher frequency in patients with less severe, seronegative, nonerosive disease. Inheriting two copies of alleles expressing the consensus sequence increases disease penetrance, time of onset, and severity. Thus, distinct genotypes can manifest as distinct clinical entities.

On the basis of early observations, two principal models were proposed to account for the association between RA and the consensus DR β-chain sequence. Both were based on the assumption

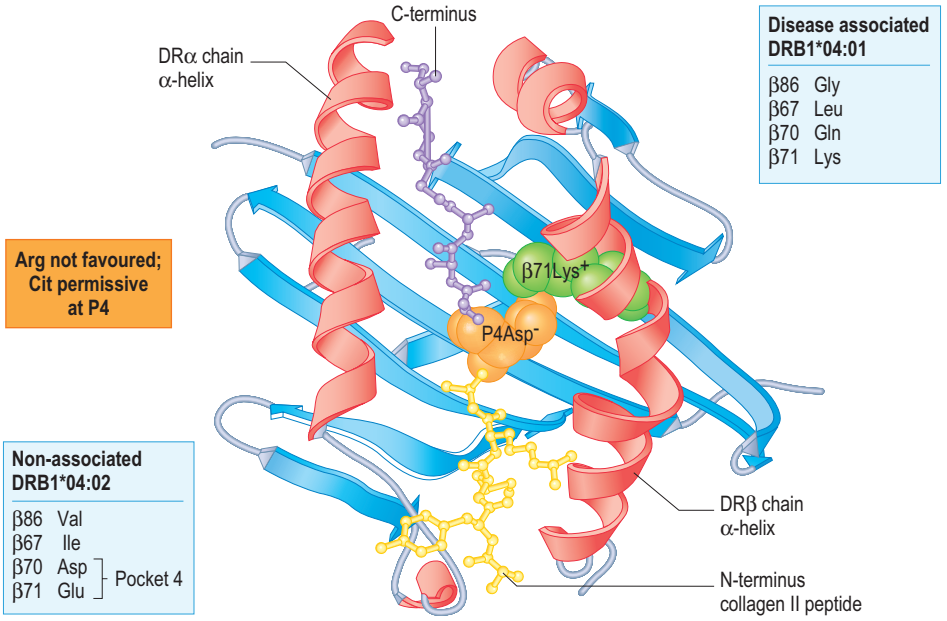

FIG. 53.1 Crystal Structure of a Collagen II Peptide/Human Leukocyte Antigen (HLA)-DR4 Complex. Ribbon model of an immunodominant collagen II peptide (1168 to 1180) complexed to HLA-DR4 (*DRA**01:01/*DRB1**04:01); a view of the major histocompatibility complex/peptide complex as seen from the T-cell surface. DRα and DRβ chain helices are shown in red, whereas the β-pleated sheet comprising the floor of the peptide-binding groove is shown in blue. Here, amino acids 11, 13, 71, and 74 form part of the fourth anchoring peptide-binding pocket, the same positions that confer highest risk of disease. Residues 67 to 74 of the DRβ chain, components of the third hypervariable region, derive the "shared epitope." The ball and stick model of the CII peptide is shown. Interacting residues of the peptide position 4 (Asp, orange) and DRβ chain residue (β71Lys, green), which make up part of pocket 4, are depicted as van der Waal's spheres. Differences in amino acid sequence between the closely related disease-associated DRB1*04:01 and non-associated *DRB1**04:02 gene products are illustrated. Note that although Arg would not be favored at position 4 in the peptide, modification of Arg → Cit by deimination would be permissive. These findings point to SE associating not with RA per se, but with an immunological phenotype, in this case autoantibodies to modified protein antigens (AMPA). Figure generated by R. Visse and A. Cope, based on crystal data derived by Wiley and colleagues (Courtesy Dessen A, Lawrence CM, Cupo S, Zaller DM, Wiley DC. X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with a peptide from human collagen II. *Immunity*. 1997;7(4):473–481).

that the SE is the critical genetic element linked directly to disease. The first model proposed that the SE determines specific peptide binding and that "pathogenic" peptides bind preferentially to disease-associated HLA class II molecules (see Fig. 53.1). This model predicted that a gradient of affinities of disease-inducing peptide for MHC class II molecules might account for the differences in susceptibility and/or severity conferred by different HLA-DR molecules. Along the same lines, disease-associated alleles may preclude the binding of peptides required for the generation of naturally occurring Tregs specific for self-peptide antigens. The second model proposed that the SE influences T-cell receptor (TCR) recognition by binding and selecting autoreactive T cells during thymic maturation and expanding these populations in the peripheral compartment; again perturbations of a repertoire of Tregs could arise through opposing influences of the SE sequence. Based on crystal structures, both models hold up, with the shared epitope sequence conferring dual functionality by determining the repertoire of specific peptides for presentation and providing a determinant for TCR recognition.

Another important line of evidence pointing to specific functions of SE^+ alleles has arisen through analysis of autoantibodies in RA patients typed at the HLA-*DRB1* locus. These studies, replicated in European and US cohorts, demonstrated associations between SE frequencies and antibodies to cyclic citrullinated peptides (anti-CCPs), as distinct from rheumatoid factor (RF). When compared with healthy controls, the odds ratios for the association between one or two copies of the SE and anti-CCPs positivity was 4.4 and 11.8, respectively. The mechanism underlying this association is illustrated in Fig. 53.1, and discussed further below. The importance of citrulline as a molecular feature of human T-cell–specific autoantigenic determinants is further suggested through identification of autoreactive $CD4^+$ T lymphocytes by flow cytometry using HLA-citrullinated-peptide tetramer complexes.[11] These tools permit determination not only of the relative proportion of antigen-reactive effector T cells, but also regulatory T-cell subsets.

Meta-analyses and fine-mapping studies, including custom-designed single nucleotide polymorphism (SNP) arrays have identified more than 100 susceptibility loci with genome-wide significance, many of which have been validated in RA populations of diverse ancestry, and numerous small effect causal variants.[12] Among the strongest associations outside HLA is *PTPN22*, initially identified in candidate gene association studies. The *PTPN22* gene variant confers risk with odds ratios ranging from 1.5 to 1.9 in Caucasian and European populations, and it is unusual among most genetic variants in that it is a coding frame mutation (R620W). *PTPN22* encodes a hematopoietic cytoplasmic protein tyrosine phosphatase whose substrates include Src and Syk family kinases, CD3ε, TCRζ, and signaling intermediates such as Vav. These signaling intermediates operate downstream of antigen receptors, integrins, and pattern recognition receptors, and so the genetic variant that alters phosphatase function has become a highly plausible susceptibility allele from an immunobiological perspective. Indeed, studies in *Ptpn22* mutant mice have demonstrated that loss of PTPN22 function perturbs activation of dendritic cells and uptake of, and presentation to, T-cells of immune-complex-derived antigens. This, together with enhanced LFA-1 dependent cell adhesion at the immunological synapse,[13] and augmented antigen receptor signaling, places dysregulation of the initiation of adaptive immune responses firmly at the center of autoimmune susceptibility linked to *HLA* and *PTPN22* polymorphisms.

Scrutiny of other, non-MHC susceptibility loci point to genes whose products are involved in proximal signaling pathways that regulate T-cell activation, differentiation, and persistence. Besides *HLA* and *PADI4*, which influence the molecular determinants of T-cell "input signals," these include *CD28, CTLA-4*, and *CD2-CD58* (regulation of T-cell costimulation); *CD247, PTPN22, PRKCQ, TAGAP*, and *REL* (transducer modules of TCR signaling); *STAT4* and *TNFRSF14* (inducers of lineage-specific cytokine gene expression and persistence of memory T cells); and *REL, IL2-IL-21, IL2RA*, and *IL2RB* (regulators of IL-2 gene expression and IL-2R signaling). Notable overlap with these and other allelic variants has been reported in other autoimmune diseases, indicating that susceptibility to RA is linked to fundamental perturbations of immune tolerance. Variants mapping to cell surface receptors (*IL6R, CCR6, CD40, CD5, FCGR2A*) and intracellular signaling intermediates (*TNFAIP3, TYK2, TRAF1, TRAF6, RASGRP1, BLK*) are well represented, as are transcription factors linked to cell differentiation and effector function (*GATA3, IRF5, IRF8, IKZF3, RBPJ, RUNX1*). Perturbations in expression or function of these genes will influence the function of a broad range of immune cell subsets.

The identification of disease-associated genetic variants raises questions about precisely how they alter cellular function, and in which cell types. This is a challenge, not least because 90% of genome-wide association studies (GWAS) associations reside in non-coding sequences, and means that gene-targeting approaches to reveal functions in mammalian cells is not trivial. Nonetheless, identification of expressed quantitative trait loci (eQTL) has supported the view that a proportion of variants alter gene expression. For example, RA genetic studies have shown that SNPs are overrepresented in memory $CD4^+$ T-cell regulatory elements. Understanding cell-type-specific gene expression enrichment in GWAS risk loci remains a topic of considerable interest.

Synovial Pathology

RA targets diarthrodial joints, structures characterized by hyaline cartilage lining opposing articulating surfaces and a cavity of viscous synovial fluid lined by synovial membrane lacking a basement membrane but encased by a fibrous joint capsule. Normal synovial tissue comprises a lining layer, no more than a few cells in depth, of stromal fibroblast-like synoviocytes (FLSs—also known as type B synoviocytes) and sublining macrophages (type A synoviocytes). The normal synovium serves to line non-cartilaginous surfaces, and although blood vessels are sparse, it functions to provide essential nutrients to avascular structures including cartilage, tendons, and bursae.

Increased Vascularity and Cell Migration

The range of pathology observed in patients with RA perhaps most convincingly underscores the heterogeneity of the disease.[14] The earliest changes observed relate to increases in vascularity characterized by vascular congestion and thrombosis with obliteration of small vessels in association with perivascular inflammatory infiltrates. Hyperplasia of the synovial lining layer is another typical early finding. These changes are rather nonspecific and certainly not diagnostic.

A key checkpoint defining the switch from acute to chronic persistent inflammation is the sustained activation of microvascular endothelium, phenotypic changes in the high endothelial venules (reminiscent of tissue injury), and the concomitant upregulation of adhesion molecules such as intercellular

adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (Chapter 16). According to current thinking, the expression of chemoattractants derived from synovial stromal cells heralds the rolling, adhesion, and transmigration of mononuclear cells through endothelial barriers into the synovial membrane. It also contributes to the progressive synovial hypertrophy and hyperplasia, sometimes with villous-like projections more typical of chronic, established inflammation. Intravital imaging of synovial joints of mice injected with arthritogenic antibodies derived from the serum of *K/B* × *N* mice indicates that enhanced vasopermeability at sites destined to become arthritic is a crucial early event, at least in antibody-induced disease. This process is dependent on mast cells and neutrophils and on the release of the vasoactive amines histamine and serotonin, which contribute to heightened vascular permeability. Neovascularization promotes further influx of inflammatory cells. To what extent this is driven by the hypoxic environment is not entirely clear, but expression of angiogenic growth factors such as vascular endothelial growth factor (VEGF), angiopoietin, Tie-2, and hypoxia inducible factor (HIF), as well as lymphangiogenic factors VEGF-C and VEGF-R3, are increased. The abundance of lymphatic vessels in inflamed synovium, suggested by expression of podoplanin and CD31 *in situ*, suggests that active lymphangiogenesis exists that may promote efflux of cells and fluid from the synovium.[14]

Organization of Lymphoid Tertiary Microstructures

Tissue microstructure both dictates and facilitates immune responses in secondary lymphoid organs and mucosa-associated lymphoreticular tissues (MALTs). These structures have evolved to coordinate responses to pathogens and to direct lymphocyte recirculation, and although their role in immune homeostasis is established, quite how they contribute to pathological states is less well understood. Thus, the inflamed, noncapsulated synovium appears to be uniquely suited to supporting distinct patterns of cellular infiltrates, including inducible lymphoid structures that promote pathways of cell activation, differentiation, and survival.[14] These include diffuse, rather disorganized lymphocytic infiltrates that comprise the most common form of synovitis, occurring in ~30% in prospective cohort studies; up to 70% has been described in late-stage disease (at arthroscopy, joint replacement surgery). In 40% to 50% of patients more organized follicular structures may exist (Fig. 53.2). Based on immunohistochemical analysis, approximately 25% of these follicular structures include organized germinal centers in which there are zones of proliferating B cells with affinity maturation, in addition to a distinct T-cell zone. In aggregates lacking germinal centers, follicular dendritic cells (DCs) are absent.

A fourth histological pattern, characterized by granulomatous reactions, has been described in a much smaller subset of patients. The cellular and molecular determinants of these structures include the homeostatic lymphoid chemokines CXCL13 and the CCR7 ligands CCL21 and CCL19, VCAM-1$^+$ICAM-1$^+$LTβR$^+$ mesenchymal-organizer cells, and hematopoietic-derived CD3$^-$CD4$^+$IL-7R$^+$RANK$^+$ lymphoid-inducer cells. Lymphoid-tissue-inducer cells produce LT-β, which is required for high endothelial venule differentiation, amplification of chemokine expression, and development of the stromal architecture. Documentation of class-switch recombination and somatic mutation of immunoglobulin genes *in situ* is further evidence of active adaptive immunity within synovial lymphoid follicles and suggests that supporting *in situ* production of pathogenic antibodies is of considerable pathobiological importance.

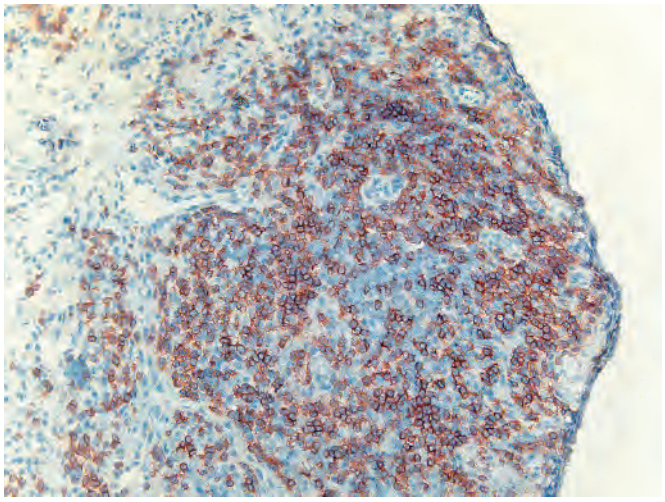

FIG. 53.2 Lymphoid Follicular Structures in Inflamed Rheumatoid Arthritis Synovial Tissue. A characteristic hematoxylin and eosin–stained tissue section from a patient with active RA showing a large follicular-like structure (original magnification ×100). This section is also stained with monoclonal antibodies to CD3ε, followed by a three-step immunoperoxidase staining protocol (CD3$^+$ T cells stained dark red). (Courtesy Tak PP, Taylor PC, Breedveld FC, Smeets TJ, Daha MR, Kluin PM, Meinders AE, Maini RN. Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum.* 1996;39(7):1077–1081, with permission from J. Wiley and Sons, Inc.)

Functional correlates of these dynamic structures continue to emerge. Using conventional histology, immunohistochemistry, and RNA-seq on synovial tissue from early RA patients it has been possible to identify three broad groups (designated pathotypes) based on cell-specific gene modules. These bear similarities to those outlined above, and are described as fibroblastic pauci-immune pathotype, macrophage-rich diffuse-myeloid pathotype, and a lympho-myeloid pathotype characterized by infiltration of lymphocytes and myeloid cells.[15] Importantly, these phenotypes map to disease severity and clinical outcomes since pro-myeloid inflammatory synovial gene signatures correlate with clinical response to initial drug therapy, whereas plasma cell genes identified a poor prognosis subgroup associated with ultrasonographic synovial thickening and increased power Doppler scores, and progressive structural damage. In addition, a more inflammatory synovial phenotype at baseline correlated with a greater fall in DAS28-CRP after 6 months of disease-modifying anti-rheumatic drugs (DMARDs) treatment, while a robust type I interferon (IFN) response in peripheral blood was associated with synovial B-cell infiltration.

Immunobiology of Rheumatoid Arthritis

Initiation of the Immune Response

Synovial fibroblasts are exquisitely sensitive to inflammatory cytokines such as IL-1, TNF, and IL-6. Fibroblast-like synoviocytes (FLSs) also express a range of toll-like receptors (TLRs) that can respond to exogenous, pathogen-associated molecular

patterns (PAMPs) and a growing range of self-tissue proteins, some of which could be considered damage-associated molecular patterns (DAMPS). Endogenous ligands especially relevant to inflammatory arthritis include heat shock proteins, fibrinogen fragments, antibody-DNA complexes, high-mobility group box (HMGB)-1, and hyaluronan oligosaccharides. Stimulation of FLS through these pathways induces cytokines such as IL-6, matrix metalloproteinases, adhesion molecules, and an array of chemokines including granulocyte chemotactic protein (GCP)-2, RANTES, monocyte chemoattractant protein (MCP)-2, IL-8, growth-related oncogene-2, and, to a lesser extent, macrophage-inflammatory protein 1α, MCP-1, EXODUS, and CXCL-16. This creates an inflammatory niche for recruiting and sustaining leucocytes in the synovial joint.

Cell-surface phenotyping of synovial FLS by mass and flow cytometry has uncovered seven subsets based on expression of podoplanin, cadherin 11, and Thy-1, segregated on the basis of the hematopoietic marker CD34.[16] In-depth transcriptomic profiling identified three broader subsets, discrete from OA synovial FLS. While $CD34^+$ fibroblasts are observed in both superficial lining and deeper sublining areas, $CD34^-Thy1^+$ fibroblasts in RA form a discrete perivascular zone surrounding capillary structures in the deep sublining layer of the synovium, especially near accumulations of lymphocytes. $CD34^-Thy1^-$ fibroblasts were mostly observed in the lining area. Synovial tissue from active, clinically swollen joints had fewer $CD34^-Thy1^-$, more $CD34^-Thy1^+$, and more $CD34^+$ fibroblasts, and the proportion of $CD34^-Thy1^+$ fibroblasts positively correlated with the proportion of infiltrated leukocytes determined by flow cytometry. These expanded, putative pathogenic populations are more proliferative and express genes associated with a migratory response, and expression of inflammatory cytokine genes such as IL6, CXCL12, and CCL2 that support matrix invasion, immune cell recruitment, and osteoclastogenesis. Adoptive transfer experiments in mice have demonstrated that two anatomically discrete and functionally distinct populations of fibroblast-activation protein alpha (FAPα) expressing FLS segregate based on Thy1 expression; $Thy1^+$ FLS are effector cells largely confined to the sub-lining layer, while the lining layer $Thy1^-$ subset express *Ccl9* and *Tnfsf11*, both potent inducers of osteoclast activity, as well as *Mmp3*, *Mmp9*, and *Mmp13*, and promote cartilage destruction.[17]

Dendritic cells (DCs) are thought to be the most important antigen-presenting cells in RA. Indeed, the proinflammatory environment favors DC maturation in regional lymph nodes as well as inflamed tissue. Thus in peripheral blood, DC precursors express either an immature $CD33^{dim}CD14^{dim}CD16^-$ phenotype or a more mature MHC class II^{bright} $CD11c^+CD33^{bright}CD14^{dim}$ surface phenotype typical of conventional myeloid DC (mDC); neither population expresses costimulatory molecules. In contrast, synovial fluid and tissue DC subsets resemble mature peripheral blood cells; in addition, a subset expresses high levels of CD86 that can support allogeneic mixed leukocyte reactions. More recent data indicate that they may differentiate further *in situ* as suggested by nuclear translocation of RelB in DC localized within perivascular infiltrates, consistent with prior cytokine receptor or Toll-like receptor (TLR) engagement *in vivo*. Perivascular RA synovium also contains populations of MHC class $II^+CD11c^-CD123^+$ plasmacytoid DC (pDC); in contrast to the conventional myeloid DC subset, these are $RelB^-$ and comprise ~30% of all synovial DC. A subset of pDC express BDCA2, capable of producing IFN-α *in situ*. Unlike their peripheral blood counterparts, synovial pDC efficiently activate allogeneic T cells to proliferate as well as to produce IFN-γ, TNF, and IL-10.

Although the common myeloid precursor cell is the precursor for all myeloid cells, including DC and tissue macrophages, the precise role of monocytes—namely $CD14^+CD16^-$, $CD14^+CD16^+$, and the more recently described $CD14^{dim}CD16^+$ subset—in synovial inflammation is uncertain. They are good candidates as persistence factors through their capacity to activate and polarize T-cell subsets, to respond to the environment through TLR expression, and to produce a wide range of inflammatory mediators, including IL-1, TNF, IL-6, IL-8, CCL2, NO, and type I IFN.

It turns out that not all myeloid cells are inflammatory. A comprehensive spatiotemporal analysis of the composition, origin, and differentiation of subsets of macrophages within healthy and inflamed joints of mice using fate-mapping approaches, fluorescence microscopy, and single-cell RNA sequencing has identified a population of CX_3CR1^+ tissue-resident, lining synovial macrophages with barrier functions typical of epithelial cells.[18] This population was derived from a proliferative subset of CX_3CR1^- cells in the deeper layers of the synovium, which acquire transcription factors for terminal differentiation into the CX_3CR1^+ lining subset, and genes with immune regulatory functions for clearance of apoptotic debris, and genes encoding tight-junction proteins. Similar populations have been defined in human synovium expression genes associated with gate keeping functions. In the serum transfer model of arthritis, inflammation-associated barrier breakdown occurred after the deposition of autoantibody-containing immune complexes.

The initial wave of inflammation has two major consequences. First, inflammatory cytokines will promote the activation of vascular endothelium, changes that occur very early in disease (see above and Fig. 53.3).[19] Under the influence of locally generated cytokines and chemokines, synovial postcapillary venules undergo morphological changes to an extent that they resemble high endothelial venules similar to those observed in secondary lymphoid organs. The second major consequence is the migration of inflammatory leukocytes, including polymorphonuclear leukocytes and immature or undifferentiated monocytes, orchestrated by chemokines produced by resident stromal as well as infiltrating cells (see Fig. 53.3). CXC, CC, C, and CX_3C chemokines all play a role, exerting chemotactic activity toward neutrophils, lymphocytes, and monocytes but also influencing the topology of inflammatory infiltrates. Besides the homeostatic chemokines described above, the key players include IL-8/CXCL8, RANTES/CCL5, MIP-1α/CCL3, SDF-1/CXCL12, IP-10/CXCL10, and MCP-1/CCL2. Upregulation on endothelium of cell-surface adhesion molecules, including ICAM-1, VCAM-1, and E-selectin, permits the rolling and adhesion of leukocytes as they migrate. In synovial joints, resident stromal cells and infiltrating macrophages are a dominant source of such factors. Crucially, the expression of cognate chemokine receptors such as CCR4, CCR5, CCR6, CXCR3, and CX_3CR1 on inflammatory cell subsets contributes selectivity of cellular recruitment.

Autoantigens in Rheumatoid Arthritis

Although current models of adaptive immune responses would suggest that DC carry antigens derived from damaged or dying synovial tissue, the molecular nature of disease-associated antigens has, until recently, remained an enigma. Many RA-associated

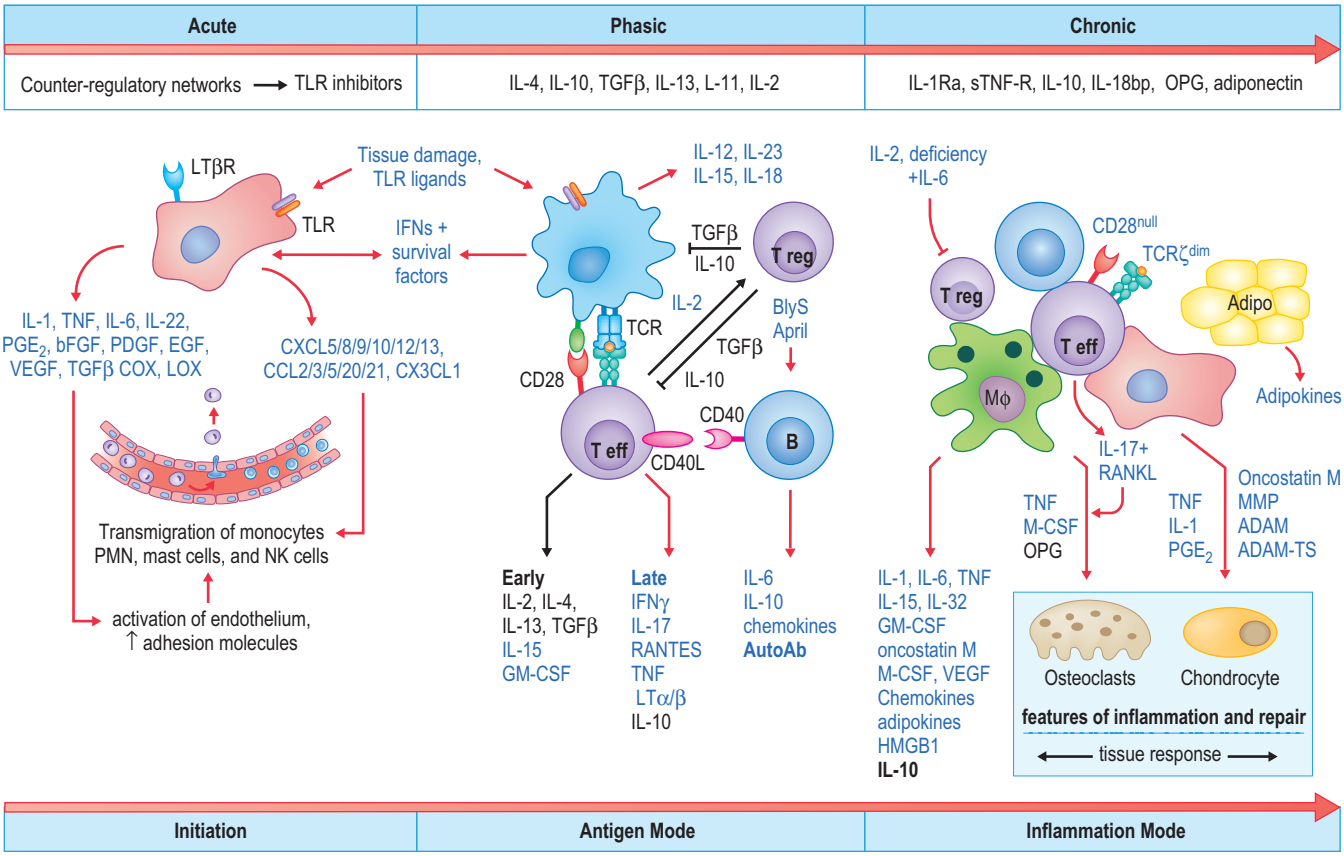

FIG. 53.3 Cytokine Networks in Rheumatoid Arthritis. The pathogenesis of rheumatoid arthritis can be thought of as a series of complex and closely related pathways temporally and spatially regulated. These include (1) an acute insult that may trigger the disease, characterized by stimulation of FLSs by inflammatory stimuli and the generation of cytokines and chemokines that promotes the migration and infiltration by cells of the innate immune system; (2) repeated episodes of antigen-specific adaptive immune responses (in lymph node, bone marrow, and *in situ*). Failure to resolve adaptive immunity is a key checkpoint that may lead to (3) a cytokine-driven chronic inflammatory phase when multiple cellular and molecular components sustain the response. Through multiple pathways acting on many cell types, this process leads to tissue injury. Proinflammatory pathways are shown in blue (text) and red (arrows), whereas anti-inflammatory, counterregulatory pathways are shown in black (text and arrows). *Adipo*, Adipocyte; *AutoAb*, autoantibodies; *B*, B cell; *DC*, dendritic cell; *FLSs*, fibroblast-like synoviocytes; *MΦ*, macrophage; *TCR*, T-cell antigen receptor; T_{eff}, effector T-helper cell; *Treg*, regulatory T cell.

autoantigens have been described (Table 53.1 for examples), and for some there exist clear correlates linked to *in vivo* arthritis models. The best described are collagen II, proteoglycans, HCgp-39, glucose-6-phosphate isomerase, α-enolase, vimentin, and citrullinated fibrinogen. However, when used as recombinant native antigen, few have been found to elicit reproducible and/or robust PB or SF T- or B-cell responses in a significant proportion of patients, when compared to healthy donors. There are several plausible explanations for this. Perhaps the most obvious is that the autoantigens used to test lymphocyte reactivity in vitro do not carry the posttranslational modifications (i.e., the neoepitopes) recognized by autoantibody or antigen receptor.

The Discovery of Citrulline as a Key Target for Autoimmunity in Rheumatoid Arthritis

In 1998, van Venrooij and colleagues first reported that patients with RA carried serum autoantibodies that recognized deiminated peptides of fillagrin.[20] Using new-generation anti-cyclic citrullinated peptide (anti-CCP) based assays, the presence of these antibodies, now collectively termed ACPAs, is now confirmed to be both sensitive (up to 80%) and highly specific (>95%) for the diagnosis of RA. Indeed, serum anti-CCPs levels are stable in established disease, can be detected many years before disease onset, and have been shown to be predictors of radiographic progression. Changes in isotype usage and spreading of antigenic specificities suggest that ACPA responses mature before disease onset. While citrullination is not specific for RA, it may be inflammation specific, having been documented in inflamed synovium from patients with reactive arthritis and psoriatic arthritis as well as RA, but not osteoarthritis (OA). What appears specific for RA is the immune response to citrulline (Fig. 53.4). Initially, linkage analysis across chromosome 6 documented a peak with logarithm of odds (LOD) scores in excess of 10 for $ACPA^+$ patients, but not for those who do not carry these antibodies. This relationship was independent of RF status because the SE allele frequencies in $ACPAs^+$ patients were twice those of $ACPAs^-$ patients, even for those patients who are RF^+.

An emerging model proposes that SE^+ DRB1 alleles are not involved in the initial breach of tolerance, but play a role in boosting the immune response to citrullinated proteins, hence

TABLE 53.1 Autoantigens in Rheumatoid Arthritis

Established	T or B Cell[a]	Molecular Specificity	Assay[b]
Immunoglobulin G	B	Human Fc IgG	Rheumatoid factor
Cyclic peptides	T and B	Citrullinated peptides	Anti-CCPs
Various peptides	B	Carbamylated peptides	Research[b]
Various peptides	B	Acetylated peptides	Research[b]
Fibrinogen peptides	T and B	Citrullinated α- and β-chain epitopes	Research[b]
Enolase peptides	T and B	Citrullinated CEP-1 peptide	Research[b]
Vimentin peptides	T and B	Citrullinated vimentin peptides	MCV assay
Collagen II	T and B	Multiple epitopes, including glycosylated epitopes	Research[b]
HnRNPA2	B	Multiple epitopes	Research[b]
Aggrecan	T and B	Citrullinated epitopes	Research[b]
HCgp-39	T	Multiple epitopes	Research[b]
Glucose-6-phosphate isomerase	B	Multiple epitopes	Research[b]

[a]Denotes autoantigens recognized by T or B cells, or both.
[b]Assay is either not commercially available or not in routine clinical use. Details of assays may be found in primary research communications.
CCPs, Cyclic citrullinated peptides; *MCV*, modified citrullinated vimentin.

the strong association between SE$^+$ DRB1 alleles and ACPA$^+$ arthritis. This concept is further reinforced by crystallographic studies suggesting that the conversion of positively charged arginine at key residues in antigenic peptides from candidate autoantigens such as fibrinogen to neutral citrulline is permissive for peptide binding and recognition by autoreactive T cells *in vivo*.[11]

Citrullination is widespread in multiple tissues in response to appropriate provocations. Although the molecular basis for these triggers is poorly understood, recent data point to a link between smoking—an environmental exposure known to be linked with RA—citrullination, and individuals carrying SE.[21] Thus, cells derived from bronchoalveolar lavage from smokers, but not from nonsmokers, express citrulline. The association between the development of RA and smoking has now been linked to ACPAs$^+$ patients, whose relative risk increases 20-fold if they smoke and carry two copies of the SE$^+$ DRB1 alleles; in comparison, the relationship in ACPAs$^-$ patients appears to be much weaker or nonexistent. A second example relates to the fact that *Porphyromonas gingivalis*, a pathogen associated with severe periodontitis, expresses its own deiminating enzyme and has the capacity to modify host proteins such as fibrinogen and α-enolase in inflamed gingival tissue. Molecular mimicry is suggested by the finding that anti-α-enolase autoantibodies from patients with RA cross-react with *P. gingivalis*–derived α-enolase. The links between autoantibodies to the immunodominant α-enolase epitope CEP1, smoking, SE$^+$DRB1, and disease-associated *PTPN22* alleles are some of the strongest defined to date, and show direct links between environmental exposure and disease-specific immune responses governed by immune-response genes.

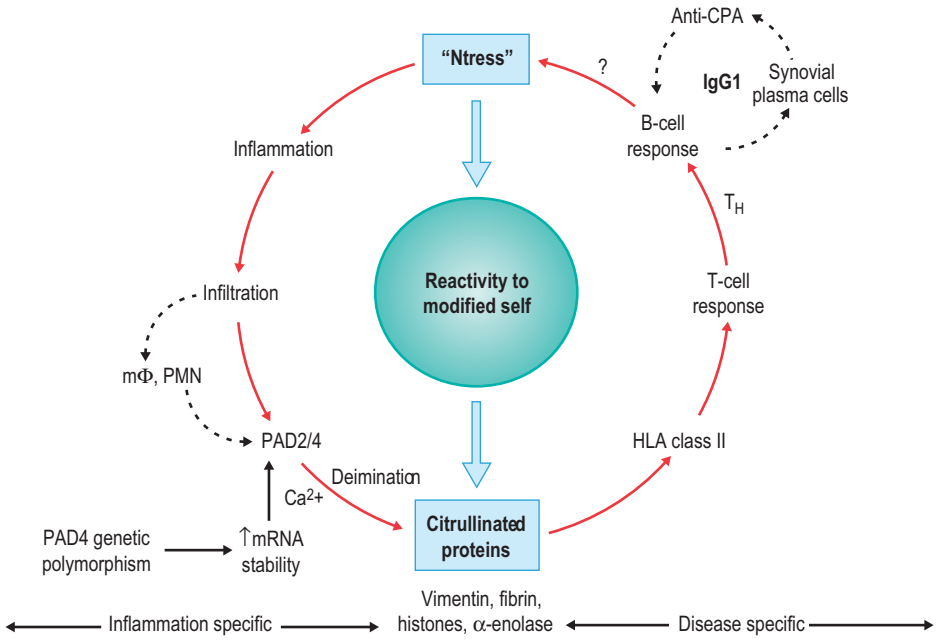

FIG. 53.4 The Generation of Autoantibodies to Citrullinated Protein Antigens. The stressed and inflamed synovium is characterized by an influx of inflammatory cells, including macrophages and neutrophils that express peptidyl-arginine deiminases (PAD). In the presence of sufficient Ca^{2+}, PADs deiminate target proteins including, among others, vimentin, fibrinogen, aggrecan, type II collagen, histones, and α-enolase. This reaction is inflammation, but not disease specific. The combination of environmental stimuli (including inflammation and exposure to tobacco smoke) and the inheritance of specific HLA-DRB1 alleles favor T- and B-cell immune responses to the host's derivatized neoepitope peptide antigens. Neoepitopes may also be generated by changes in peptide cleavage during antigen processing as a consequence of the Arg → Cit modification. *Anti-CPA*, Autoantibodies to citrullinated protein antigens; T_H, T-helper effector cell.

Our growing appreciation of the importance of post-translational modifications (PTMs) in general, and citrullination in particular, has driven a search for alternative PTMs that might contribute to the triggering or perpetuation of autoimmune responses to altered self.[22] For example, autoantibodies recognizing peptides carrying amino acids modified by carbamylation (to homocitrulline) or acetylation (to acetyllysine) are readily detectable, collectively termed anti-modified protein antibodies (AMPA). Mapping antibody reactivities against multiple peptide specificities indicates that citrulline-specific B cells are highly cross-reactive—a feature that likely underpins the basis of breech of immune tolerance prior to the onset of RA, and supported by findings from mouse immunization experiments revealing that different AMPA responses emerge from exposure to a single type of modified protein.

Lymphocyte Biology

Flow cytometric analysis of dissociated synovial mononuclear cell cultures indicates that infiltrating T lymphocytes make up approximately 10% to 35% of cells in inflamed tissue. Synovial T cells express phenotypic markers of antigen experienced, terminally differentiated T cells with enhanced migratory capacity. Thus, synovial T cells typically carry cell surface markers such as HLA-DR$^+$, LFA-1$^+$, VLA-1$^+$, CXCR3, CD69$^+$, CD45RO$^+$, CD45RA$^-$, CD45RB^{dim}, CD29^{bright}, CD27$^-$, and CD25$^-$, and low expression of TCRζ. While synovial fluid T cells are also FasL$^+$, Bcl2$^-$, Bax^{bright}, favoring a proapoptotic state, it is thought that environmental cues transduced through common γ chain receptor signaling cytokines such as IL-2, IL-7, and IL-15, as well as type I interferons, prevent apoptosis of T cells *in situ*. Synovial tissue–derived lymphocytes may be different. Consistent with their state of terminal differentiation, a subset of synovial T cells are CD28$^-$, while at the same time expressing a range of natural killer (NK) cell-surface receptors that are thought to contribute to effector function independently of cognate antigen.

Recent analysis of citrulline-reactive B cells from RA patients indicate that they display markers of class-switched memory B cells and plasmablasts (CD20$^+$CD27$^+$IgD$^-$). Analysis of the variable (V) regions of the immunoglobulin heavy and light chains confirm that antigen-specific activation and differentiation of B cells into plasma cells takes place in draining lymph nodes as well as in the chronically inflamed synovial tissue of patients with RA. Likewise, analysis of the T-cell repertoire indicates that the synovium provides a niche for supporting expansion of specific T cells, whose clonality is shared between different joints from the same patient, but not substantially with clones from paired blood samples.

For over a decade it has been known that one of the dominant cytokines expressed in synovial T cells from patients with established disease is IFN-γ. Somewhat surprisingly, a significant proportion of IFN-γ$^+$ cells also express IL-10. Recent data support a model in which there exists, during differentiation, a transition from IFN-γ$^+$ Th1 T cells through an IFN-γ$^+$IL-10$^+$ double-positive stage to a single-positive IL-10$^+$ stage. This last phase could represent part of the normal life cycle of an effector T cell, where IL-10 expression promotes the resolution of the adaptive immune response, attenuating the function of DCs. Interestingly data indicate that this Th1 life cycle involving a switch from IFN-γ to IL-10 production may be defective in individuals at risk of developing RA.

Recent, systematic approaches for studying synovial T cells at a single cell level has uncovered a markedly expanded population of PD-1^{hi}CXCR5$^-$CD4$^+$ T cells in synovium of patients with RA.[23] These are unusual in light of high expression of PD-1 and lack of CXCR5, and while they express activation markers such as MHC class II, they are not exhausted. Defined as peripheral helper T cells (T_{PH}), and distinct from T_{FH} cells, synovial PD-1^{hi}MHCII^{hi}CXCR5$^-$ cells support B-cell help via expression of IL-21, CXCL13, ICOS, MAF, and BLIMP1, and have been shown to induce plasma cell differentiation at least *in vitro*.

Finally, PTM of immunoglobulins, most notably glycosylation of the Fc domain, has been a recognized feature of the evolving immune response for decades, with distinct moieties being linked to specific IgG effector functions. Besides glycosylation at position 297 in the IgG Fc tail of APCAs, where animal studies suggest that disease-associated glycosylation patterns are regulated by IL-23 and Th17 cells, a recent addition to the repertoire of glycosylation of ACPAs has been the identification of highly sialylated N-linked glycans in the antigen variable domain of up to 90% IgG ACPA, arising as a consequence of T-cell dependent, variable region somatic hypermutation.[22] This is rather uncommon in other IgG molecules. Analysis of IgG ACPA in first-degree relatives of RA patients suggests that extensive glycosylation precedes the onset of disease, and thus represents a signature of high risk. While Fc tail agalactosyl "pro-inflammatory modifications" likely alters immunity through effects on FcR binding and effector responses, current thinking suggests that ACPA IgG variable domain N-glycans could influence antigen binding avidity, B-cell receptor signaling and tolerance, or binding to glycan receptors such as lectins.[22] Further work is needed to evaluate each of these possibilities.

Immune Regulation

Investigation of regulatory cell subsets and their anti-inflammatory properties has perhaps more firmly established the concept that failure of the host's intrinsic mechanisms of immune regulation can underpin autoimmune diseases. Experiments in gene-deficient mice (*e.g.*, Foxp3, IL-2, IL-2R, IL-2R signaling, STAT5, IL-10, TGF-β) lend support to this concept. In RA the data remain less clear. For example, there is *in vitro* evidence for a relative deficiency of constitutive IL-10 expression in synovial cell cultures, and yet clinical trials of IL-10 have been disappointing. These results may reflect the complex role of these cytokines in disease pathogenesis. The identification of defective numbers and/or function of CD4$^+$CD25^{bright} Tregs has been suggested by several investigators, but reports are conflicting.[24] Some studies have shown clear reductions in numbers of peripheral blood Tregs in patients with RA, whereas others have shown no difference. In synovial joints, the data are more consistent, with many reports showing substantial increases in Treg numbers in synovial tissue and fluid compared with paired PB. However, some studies have reported normal function at a cellular level, whereas others have shown depressed regulatory function. One possible mechanism is that synovial effector T cells are refractory to regulatory pathways.

The advent of immune checkpoint inhibition (CPI) for the treatment of a wide range of cancers has, serendipitously, provided incontrovertible *in vivo* evidence that immune checkpoints CTLA-4 and PD-1 contribute to immune homeostasis in individuals at risk of inflammatory arthritis.[25] Immune-related adverse events (irAEs) are now a well-recognized complication of CPI with anti-PD-1/PDL-1 and anti-CTLA4, targeting any organ or tissue in the body. These inflammatory syndromes, which phenocopy idiopathic autoimmune diseases, can arise within weeks or months of treatment initiation, and are thought to represent an abrupt breach of immune

tolerance. Both seronegative and seropositive subsets of inflammatory polyarthritis, indistinguishable from RA, have been reported by many groups; some cases are transient and self-remitting, while others persist, requiring disease-modifying therapy. What remains unclear is whether this RA-like syndrome arises only in susceptible individuals or whether the inflammatory process arises *de novo*, regardless of genetic or environmental factors.

Impact of the Inflammatory Response on Cartilage and Bone

For many years, it was considered that the terminal effector phase of chronic inflammation that led to cartilage destruction and bone resorption was driven almost exclusively by inflammatory cytokines and proteinases. IL-1, MMPs (MMP1, 3, 8, 13), and aggrecanases (ADAMTS 4 and 5) were, and remain, major drivers. Attempts to establish more directly a link between adaptive immunity and destruction of target tissue failed, not least because of the lack of a direct physical link between lymphocytes, chondrocytes, and bone. A breakthrough came in the late 1990s with the identification of the TNF/TNFR family member receptor for activation of nuclear factor (NF)-κB ligand (RANKL)/TRANCE/ODF and its counter-receptor RANK, and the dissection of the molecular and cellular components required for osteoclast differentiation from monocyte precursors.[26] According to contemporary paradigms, RANKL is necessary and sufficient for osteoclast differentiation. TNF, M-CSF, IL-1, and IL-17 contribute, and RANKL-independent pathways may also play a role. RANKL is expressed on synovial fibroblasts and osteoblasts but also on activated T cells, its counter-receptor being expressed on myeloid lineage cells including monocytes, osteoclast precursors, and DCs. Its expression is regulated by inflammatory mediators including TNF and PGE_2. RANKL is shed, probably through the action of several membrane-associated proteases including MT1-MMP (MMP14). Gene targeting of RANKL or RANK in mice leads to inhibition of osteoclastogenesis and a profound osteoporotic bone phenotype. Deletion of osteoprotegerin (OPG), the naturally occurring decoy soluble receptor for RANK, leads to unbridled osteoclast differentiation and bone resorption and substantially reduced bone mass. In RA, several studies have demonstrated perturbations of serum RANKL/OPG ratios, and recent clinical experience with denosumab, a fully humanized monoclonal antibody that binds to RANKL, demonstrates increased bone mineral density and reduced bone turnover in patients with RA.

Distinct from enhanced bone resorption, bone formation is impaired in RA, although until recently the pathways involved were rather obscure. The inflammatory process itself, along with its effects on osteoblast maturation and function, has been directly implicated because bone formation at surfaces adjacent to bone marrow, as opposed to inflamed synovium, are relatively well preserved. Recent data suggest that ACPAs can also directly promote osteoclastogenesis, providing a mechanistic link between autoantibodies and joint destruction that also depends on IgG glycosylation status. Evidence now points to the canonical Wingless (Wnt) signaling pathway as being an essential control point in osteoblast function, based on the observation that in animal models of RA antibodies to Dickkopf homologue 1 (DKK1), a secreted antagonist of Wnt blocking, signals at the level of its cognate receptor Frizzled, promote bone formation and inhibit bone resorption indirectly by increasing production of OPG. This indicates that Wnt signals negatively regulate osteoclastogenesis.

The interplay between bone formation and resorption is fundamental to bone homeostasis, especially in the context of chronic inflammatory disease. Recent evidence suggests that the molecular basis for this so-called coupling lies with the osteoprotective factor semaphorin 3 A. This factor regulates bone mass in both osteoblasts (through effects on the canonical Wnt/β-catenin signaling pathway) and osteoclasts (by inhibiting RANKL-dependent osteoclast differentiation). Together these data support the view that bone and joint integrity are regulated by a delicate balance of catabolic and anabolic immune and inflammatory mediators influencing the maturation and function of osteoblasts (Wnt:DKK1) and osteoclasts (RANKL:OPG).

CLINICAL FEATURES

Disease Onset

RA is a heterogeneous disease that does not conform to a single clinical entity. Whereas 10% of patients may have an acute severe onset and 20% a more subacute onset, the onset of signs and symptoms may be insidious in up to 70% of patients. A more episodic or palindromic onset has also been described. A common presentation, more likely during the winter months, will be that of a female in her fifth to sixth decade of life who complains of diffuse symmetrical joint pain, swelling, and stiffness of peripheral joints. Patients may complain that they can no longer make a fist, especially in the early morning. The targeting of afflicted synovial joints may be symmetrical in most, but not all, cases, typically affecting small joints of the hands and feet as well as wrists. Less frequent are those presenting with slow-onset monoarticular disease. Patients not fulfilling the diagnostic classification criteria for RA may be ascribed the more appropriate diagnostic label of undifferentiated arthritis, because in a proportion of cases signs and symptoms may resolve spontaneously. Systemic disease is much less common in current clinical practice, in part through application of earlier, more intensive treatment regimens (see below). Nonetheless, the systemic nature of the disease can be manifested through a wide array of systemic, extraarticular clinical features that may occur in patients with disease at the more severe end of the spectrum. A spectrum of disease severity may also be evident from hand radiographs; examples are shown in Fig. 53.5.

Diagnosis

Classification Criteria

The American College of Rheumatology criteria for the classification of RA are a set of clinical and laboratory parameters, established largely for epidemiological purposes, that serve as a guide for the diagnosis of RA. They are relatively straightforward and easy to apply, especially to patients with established disease. However, failure of a patient with early signs and symptoms of an inflammatory arthropathy to fulfill them does not mean that the individual does not have RA. The 1987 criteria were simplified further by removing the "probable," "definite," and "classic" subclassifications. These criteria returned a sensitivity for RA of 91% to 94% and a specificity of 89% in the clinical setting. New criteria, established by a steering group comprising members of the ACR and EULAR and published in 2010, are based on a weighted score around four domains, including distribution and type of joint involvement (tender as well as swollen joints are included, scoring 0 to 5 points), serology (0 to 3) that includes RF or ACPAs and is weighted according to antibody

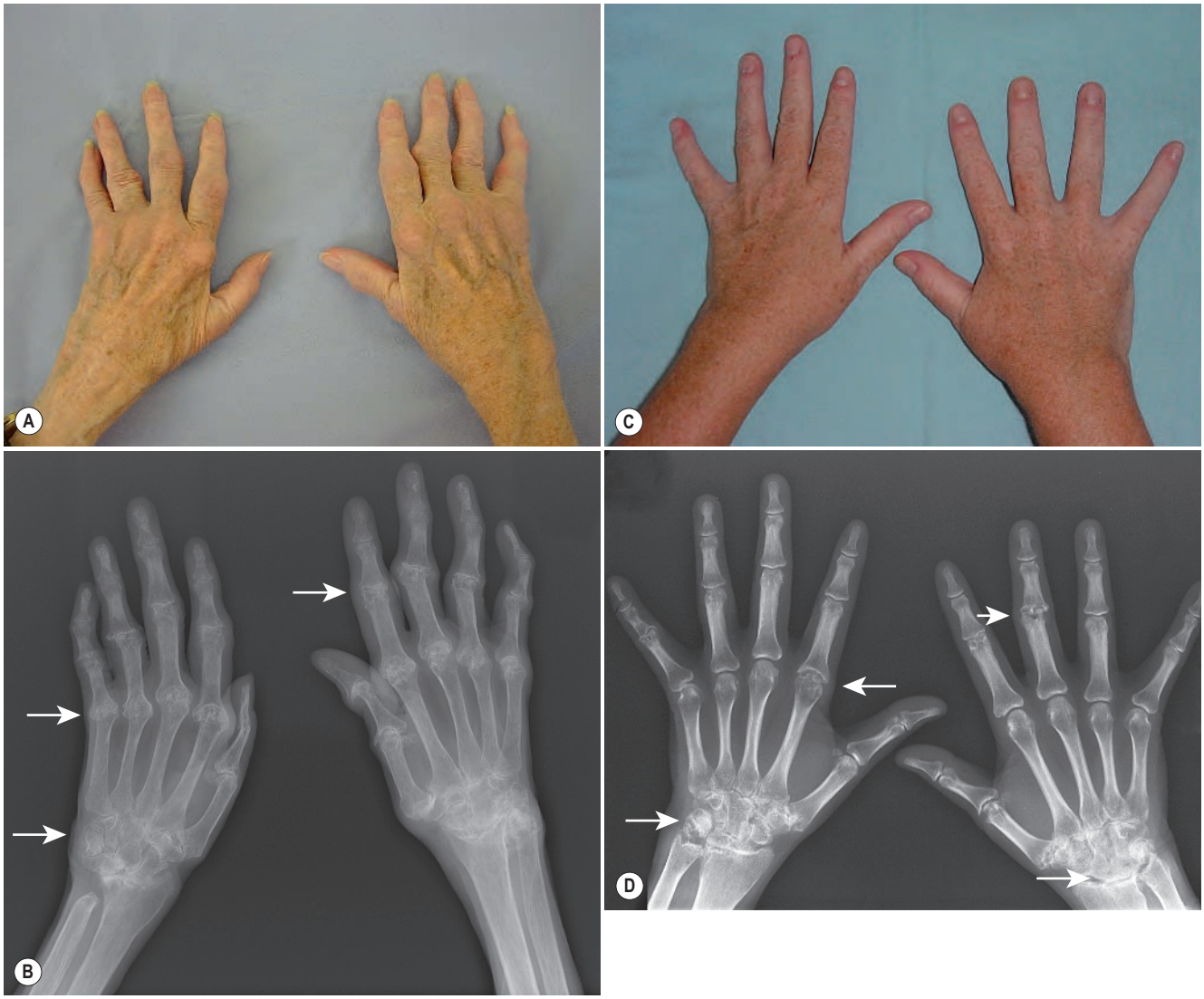

FIG. 53.5 Photomicrographs and Radiographs of the Rheumatoid Hand. Chronic, severe, and erosive RA, refractory to treatment and showing joint swelling and classical deformities (A and B), is compared with erosive disease whose progression has been attenuated by combination and biological therapy (C and D). White arrows indicate major areas of bone and cartilage destruction. (Reproduced with kind permission from the patients.)

levels, duration of synovitis (0 to 1), and acute-phase reactants erythrocyte sedimentation rate or C-reactive protein (0 to 1). A score of 6 or above is indicative of an early disease state requiring initiation of DMARDs such as methotrexate (Table 53.2), and so these criteria are thought to better reflect an intention to treat on the part of the supervising physician. Although the presence of synovitis in at least one joint is required (in the absence of an alternative diagnosis that better explains the synovitis), there remains debate as to whether subclinical synovitis of specific joints, defined by magnetic resonance imaging (MRI) or high-resolution ultrasonography (HRUS), should be included in the joint score.

Laboratory Findings

Until the late 1990s, IgM RFs, autoantibodies that recognize the Fc subunit of IgG, remained one of the few parameters of value in the clinical setting, forming the basis of the seropositive versus seronegative RA stratification and identifying those patients more likely to progress to erosive disease with or without extraarticular features. Nevertheless, RF can be detected in up to 5% of the healthy population and in 10% to 20% of the elderly population (>65 years of age); RF is found in a range of rheumatic conditions including Sjögren syndrome, SLE, and cryoglobulinemia, as well as in acute infectious and neoplastic disease entities, influencing its diagnostic utility. In general RF is not of value for monitoring responses to therapy.

The discovery of ACPAs, which can be detected very early in the disease process, has had a major impact on diagnostic practice as the assays have become more widely available.[20] They also have prognostic value in terms of radiographic progression, and titers may alter with therapy. The new-generation anti-CCPs kits have demonstrated diagnostic sensitivity of 80% and specificity of 98%. As the range of RA-associated autoantigens has expanded and the repertoire of citrullinated target autoantigens has become better defined, multiplex assays of serum autoantibodies are likely to play an increasingly important role in the diagnosis and prognosis of subsets of autoantibody-positive inflammatory arthritides.

TABLE 53.2 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis

Domain	Weighted Score
Joint Involvement (0–5)	
1 medium to large joint	0
2–10 medium to large joints	1
1–3 small joints	2
4–10 small joints	3
>10 joints (with at least one small joint)	5
Serology (0–3)	
Neither RF- nor ACPAs-positive (≤ULN)	0
At least one test, low positive titer (>1 ≤3 × ULN)	2
At least one test, high positive titer (>3 × ULN)	3
Duration of Synovitis (0–1)	
<6 weeks	0
≥6 weeks	1
Acute-Phase Reactants (0–1)	
Neither ESR nor CRP abnormal	0
Abnormal ESR or CRP	1
TOTAL (≥6 indicates *definite* RA)	_____

ACPAs, Antibodies to citrullinated protein antigens; *ACR*, American College of Rheumatology; *CRP*, C-reactive protein; *ESR*, erythrocyte sedimentation rate; *EULAR*, European League Against Rheumatism; *RF*, rheumatoid factor; *ULN*, upper limit of normal.

CLINICAL PEARLS

Predictors of Poor Outcome in Rheumatoid Arthritis

- Chronic, unremitting disease onset, especially at advanced age
- High disease activity scores at baseline
- Female gender
- Poor functional status as determined by validated functional disability indices such as the Stanford Health Assessment Questionnaire (HAQ) and the Arthritis Impact Measurement Scale (AIMS)
- Low socioeconomic status
- Systemic and extraarticular features
- Depression and anxiety
- Comorbidity, for example, infection, cardiovascular disease, renal impairment
- Early erosive disease (in first 6–12 months; may be associated with ACPAs autoantibodies)
- Persistent acute-phase response (e.g., time-integrated CRP levels)
- Autoantibodies (RF and ACPAs) and HLA-DRB1 status (SE+)
- Significant delay in early use of DMARDs and corticosteroids

ACPAs, Antibodies to citrullinated protein antigens; *CRP*, C-reactive protein; *DMARDs*, disease-modifying antirheumatic drugs; *RF*, rheumatoid factor; SE, shared epitope.

The Preclinical Phase of Rheumatoid Arthritis

The identification of a preclinical phase of RA, that can be detected by virtue of serum autoantibodies up to 14 years before disease onset, has provided opportunities to characterize the RA prodrome in more detail. Work has centered around evaluation of acute-phase proteins and serum cytokines and chemokines, which appear to rise 1 to 2 years before onset of clinical disease; ACPAs isotype usage, N-glycan modifications, and epitope spreading; whole-blood gene expression analysis, which shows similarities to patients with established disease (including type I interferon-inducible gene signatures in a subset); imaging by MRI and ultrasonographic modalities; and, most recently, analysis of lymph node immune phenotypes, showing evidence of lymphocyte activation. The strongest predictors of progression in those at-risk subjects are joint pains without clinically detectable synovitis (arthralgia), combined with the presence of high-titer ACPAs and RF autoantibodies. Risk scores, which take into account other demographic factors, have been developed and are currently being validated. In 2016, a EULAR definition of the clinical characteristics of patients at risk of developing RA was published.[27]

Progression rates for ACPA+ arthralgia patients with subclinical synovitis detected by ultrasound examination are of the order of 20% over a median of 28 months,[28] while arthritis-free survival curves suggest that 40% to 50% of those at highest risk will develop clinically apparent synovitis in 2 to 3 joints within 24 months. Somewhat surprisingly, genetic markers, such as HLA-DRB1 SE, have not contributed dramatically to risk estimates, while emerging patterns of tenosynovitis by ultrasonography or MRI, or bone marrow oedema (or osteitis) by MRI define some of the earliest articular features of this at-risk state. Rates of progression are remarkably consistent across at-risk cohorts and are considered sufficient to justify secondary prevention intervention studies. Thus, in 2019 the effects of a single IV dose of rituximab were studied in ACPA+ arthralgia subjects. Compared to a control group (who also received IV hydrocortisone at baseline), rituximab delayed disease onset by about 12 months. As of spring 2020, there are three other clinical trials actively examining the impact of a fixed period of therapy with hydroxychloroquine, methotrexate, or abatacept versus placebo and followed up for a 2- to 3-year period. If delay or prevention is observed, even in a subset of individuals, this is likely to transform the way we approach the treatment of RA.

ON THE HORIZON

The Preclinical Phase of Rheumatoid Arthritis

- High-risk individuals include those with inflammatory joint pain (arthralgia) and serum RA–associated autoantibodies. From 40% to 50% of those with high-titer ACPAs (with or without rheumatoid factor) may develop clinical synovitis within 24 months.
- Targeting these high-risk individuals with preventative strategies provides the best chance of achieving cure. Trials of rituximab, abatacept, or hydroxychloroquine are ongoing.
- In-depth molecular and cellular studies for characterizing the preclinical phase of disease will be critical to the success of this endeavor.
- Models for progression to RA have identified the following phases:
  - I. Genetic risk
  - II. Genetic risk with autoimmunity (e.g., ACPAs, rheumatoid factor)
  - III. Genetic risk with autoimmunity and arthralgia (but absence of clinically apparent synovitis)
  - IV. Undifferentiated arthritis (clinically apparent synovitis, not fulfilling RA classification criteria)
  - V. Early RA (fulfilling disease criteria; need for DMARDs)
- Stratification of risk, including genetic, serological, and demographic factors, will permit the identification of subjects most suitable for intervention studies.
- Studying the impact of lifestyle modifications, for example, diet, stopping smoking, would be of great interest.
- Reestablishing immune homeostasis and/or induction of immune tolerance may provide the best chance of achieving cure in subjects during the preclinical phase of disease. Early-phase studies of peptide immunotherapy indicate that this approach is well tolerated.
- Establishing robust assays for signatures of immune tolerance (e.g., immune phenotyping by flow cytometry, extended autoantibody serotyping, multiplex assays for detection of inflammatory mediators in serum, whole-blood transcriptomic profiles) that reflect a healthy immune system will greatly facilitate these endeavors.

ACPAs, Antibodies to citrullinated protein antigens; *DMARDs*, disease-modifying antirheumatic drugs; *RA*, rheumatoid arthritis.

TREATMENT

Disease-Modifying Antirheumatic Drugs

THERAPEUTIC PRINCIPLES

Treatment Paradigms in Rheumatoid Arthritis

- Education and counseling through early involvement of a multidisciplinary team, including specialist nurse and other health-care professionals; appropriate balance of rest and exercise during disease flares
- Adequate nutrition (especially important with severe, active disease)
- Comprehensive assessment of disease activity, especially during early phase of disease to achieve rapid disease control
- Complete suppression of inflammation early in the disease, with tight control through regular and frequent reassessments focused around disease activity scores
- Yearly imaging assessments to monitor radiographic progression, for example, X-rays or high-resolution ultrasonography of hands and feet
- Early use of DMARD/SAARDs
- Early use of corticosteroids, including use of intraarticular joint injections to suppress inflammation, and use of step-up combination therapy in severe disease
- Appropriate use of biologicals, for example, early use of anticytokine, T- or B-cell–targeted therapies in severe disease
- Early relief of pain with judicious use of NSAID or COX2 inhibitors according to safety/risk profile
- Monitoring for drug toxicity
- Effective contraception, where appropriate
- Bone protection
- Monitoring for risk factors of key comorbidities, including cardiovascular disease
- Prevention of infection through vaccination (preferably before instituting immunosuppressive agents), for example, against influenza, pneumococcus, and herpes zoster

COX, Cyclooxygenase; *DMARD*, disease-modifying antirheumatic drugs; *NSAID*, nonsteroidal anti-inflammatory drugs; *SAARD*, slow-acting antirheumatic drugs.

Over the past two decades there has been a dramatic paradigm shift in the therapy of RA from control of symptoms to the control of the disease process and aggressive suppression of inflammation. This shift has come about through a growing appreciation of the relationship between joint inflammation and joint destruction, as well as the development of imaging technology for detecting the very earliest changes in joint structures. The impact of this paradigm shift in therapeutic terms is striking. Traditional "go-low, go-slow" regimens of the 1970s and 1980s included the initiation of nonsteroidal anti-inflammatory drugs (NSAIDs), followed by implementation of DMARDs only after destructive disease became evident. Depending on the clinical response, sequential monotherapy was the norm. Although this strategy may still be appropriate for patients with mild disease, current practice now dictates aggressive combination therapy (two or more conventional DMARDs) from the outset for patients with poor prognostic factors, with preference for the faster-acting DMARDs such as methotrexate, leflunomide, and sulfasalazine (onset 3 to 6 weeks) over slower-acting agents such as hydroxychloroquine, gold, and D-penicillamine (onset 3 to 6 months), but with the addition of oral or parenteral prednisolone. More recent data suggest that the specific choice of therapy may be less important than the strategy. For example, the pioneering TICORA and BeST studies both indicate that intensive treatment when combined with intensive control most convincingly influences outcome measures, including clinical response, retention, functional status, and radiographic progression. The benefits of tight control have been substantiated in many subsequent clinical trials, as well as in "real-life" clinical practice.[29]

Anti-Cytokine Therapy

The introduction of targeted therapy to the clinic using biological agents (e.g., chimeric or fully humanized antibodies to ligands or receptors, soluble receptor fusion proteins, or recombinant receptor antagonists) has transformed the treatment of RA. The prototype biologic, developed in the 1990s, was TNF blockade.[30] The rationale for inhibiting TNF bioactivity is based upon its pleiotropic effects on cell activation, cellular adhesion and migration, induction of cytokine and inflammatory gene mRNA and protein, neoangiogenesis, and the regulation of cartilage catabolic factors such as IL-1 and matrix metalloproteinases (see Fig. 53.3). TNF and other inflammatory cytokines such as IL-1, IL-6, IL-15, IL-17, and GM-CSF are expressed constitutively in inflamed synovial tissue at mRNA and protein level. In many cases the expression of their high-affinity cognate receptors is also upregulated and the functional activity of the corresponding naturally occurring inhibitors (e.g., soluble TNF-R or IL-1Ra) is reduced further, promoting persistence of the inflammatory response (although levels of protein may be increased, reflecting an attempt at restoring homeostasis).

Chimeric anti-TNF monoclonal antibodies (infliximab) were first used to treat RA in open-label clinical trials in 1992.[30] Humanized antibodies (adalimumab) and the soluble p75 TNF-R IgG fusion protein (etanercept) were tested soon after with comparable therapeutic effects; golimumab and a construct comprising a PEGylated anti-TNF antibody Fab fragment (certolizumab) are also licensed for use in patients with RA, as are a growing number of "biosimilar" TNF inhibitors. TNF-α blockade leads to dramatic and rapid reductions in symptoms (pain, stiffness, and fatigue) and signs (joint pain and swelling) of arthritis in a dose-dependent fashion, and in a significant proportion of patients (~60% to 70%) who have failed conventional DMARDs.[30] As a class, and when used in combination with methotrexate, the majority are superior to either drug alone.

The clinical benefit of TNF blockade has prompted extensive mechanism of action studies.[30] Anti-TNF reduces the acute-phase response, including IL-6 serum levels. Leukocyte trafficking is inhibited, as demonstrated through an early (within hours) and dramatic rise in lymphocyte counts through demargination, a more prolonged and sustained exclusion of leukocytes based on reductions in cellularity of synovial tissue biopsies after treatment and suppression of markers of angiogenesis, including VEGF; TNF blockade promotes lymphangiogenesis and may facilitate cell egress from inflamed synovium. TNF blockade has also been shown to downregulate markers of cartilage and bone destruction, including the collagenases MMP1 and 3, and to reduce the ratio of RANKL and OPG in serum, effects that might explain in part the joint-preserving effects of anti-TNF *in vivo*.

The IL-1 receptor antagonist (IL-1Ra) is the only IL-1 inhibitor currently licensed for use in RA. It has disease-suppressing effects in animal models of arthritis, with potent joint protection, but has proven less effective than anti-TNF in patients with RA. Nevertheless, it has been used effectively to treat patients who have failed TNF blockade, slowing radiographic progression, and may be efficacious in a subset of individuals with more systemic autoinflammatory

syndromes. Anti-IL-6R blockade (tocilizumab) is licensed for use in patients with established RA. Evidence suggests that in early disease, tocilizumab as monotherapy is as effective in suppressing signs and symptoms of RA as when it is combined with methotrexate. These effects are mediated through blocking IL-6 actions on the immune response, the acute-phase response, osteoclastogenesis, B-cell activation and immunoglobulin production, angiogenesis, and cell adhesion (reviewed in Kishimoto).[31] Clinical responses are comparable to those observed with TNF blockade, and a range of IL-6 inhibiting drugs are now licensed for use. Unlike in psoriasis, the effect of inhibiting IL-17 in RA (with humanized monoclonal antibodies ixekizumab or secukinumab that block IL-17A) has been more modest, although there may exist a subset of RA patients in whom IL-17-driven inflammatory responses dominate. A role for blocking GM-CSF is emerging.

Finally, the impact of using small molecule inhibitors of cytokine signals, transduced through receptors that utilize members of the Janus kinase (JAK) family of tyrosine kinases,[32] has been evaluated in some detail. These are interesting, immunologically important kinases, because gain-of-function mutants are associated with leukemia and lymphoma, whereas loss-of-function is associated with primary immunodeficiency. Jakinibs, including tofacitinib, baricitinib, and upadacitinib, have been studied in phase III clinical trials of RA patients. Tofacitinib has high affinity for JAK3, but is also able to partially inhibit JAK1 and, to a lesser extent, JAK2; baricitinib blocks JAK1 and JAK2 to a similar extent, with some Tyk2 targeting activity, while upadacitinib has more JAK1 selectivity. These agents are well tolerated, with acceptable safety profiles and now provide physicians with an orally available agent with efficacy comparable to biologic agents. This is likely due to the wide range of inflammatory pathways that are targeted, including common gamma chain cytokines IL-2, IL-7, IL-15, and IL-21, type I and type II interferons, and IL-6 family cytokines.

Anti-T-Cell Therapy

The contribution of costimulatory signals ("signal 2") transduced through CD28 to priming and activation of naïve T cells and amplification of cytokine gene expression and proliferative responses has provided a rationale for testing costimulatory blockade in patients. This has been achieved using a nondepleting humanized IgG1-CTLA-4 fusion protein that prevents CD80 and CD86 (expressed on antigen-presenting cells) from engaging CD28 (but also CTLA-4) expressed on T cells. Initial studies confirmed that the agent was safe and well tolerated.[33] As well as suppressing disease activity, CTLA-4-Ig (licensed as abatacept as intravenous and subcutaneous formulations) inhibits radiographic progression and structural damage and it is also effective in treating those patients who have had inadequate responses to TNF blockade as well as to methotrexate. Head-to-head studies indicate that the kinetics of response are remarkably similar to those of anti-TNF when compared as monotherapy, indicating that a significant number of costimulation-dependent T cells actively participate in the ongoing inflammatory response. Clinical improvement may continue beyond 12 months. Recent data suggest that inhibition of CD28 signals reduce the number of T_{FH}. Abatacept targets one of the earliest stages of adaptive immunity, and so offers a biologically plausible pathway to target in subjects at high risk of progressing to RA.

Anti-B-Cell Therapy

Rituximab is a humanized monoclonal antibody that recognizes human CD20, a 33- to 37-kDa membrane-associated phosphoprotein expressed on pre-B, immature, and mature B cells but not on plasma cells. It was initially developed, and then licensed, for the treatment of non-Hodgkin lymphoma. While CD20 ligation promotes B-cell activation, differentiation, and cell cycle progression, the function of CD20 is still poorly understood. The therapeutic effects of anti-CD20 are related to B-cell depletion, which can vary between patients due to antibody-dependent cell cytotoxicity, complement-mediated cell lysis, and/or triggering of intracellular pathways for apoptotic cell death.[34] The effects on serum immunoglobulin levels are modest with levels remaining in the normal range, unless patients undergo repeated cycles of B-cell depletion.

A pivotal placebo-controlled trial of rituximab therapy randomized 161 patients with RF-positive RA to compare the efficacy and safety of methotrexate alone (standard therapy) versus methotrexate plus rituximab (1000 mg on days 1 and 15), rituximab alone, or rituximab plus cyclophosphamide.[34] Up to 43% of patients receiving the combination of rituximab and methotrexate achieved 50% improvement in clinical and laboratory parameters after 24 weeks (ACR50), and this was at least as good as the rituximab/cyclophosphamide combination (41% achieving ACR50) and superior to methotrexate (13%) or rituximab alone (33%). Follow up studies indicate that B-cell repopulation occurs at a mean of 8 months after treatment, comprising immature $IgD^+CD38^+CD27^-CD5^+$ B cells, and is associated with increased serum B cell–activating factor (BAFF) levels. Early relapse is associated with reconstitution of the $CD27^+$ memory B-cell compartment.

Future Prospects for Therapy

There remain unmet needs in the treatment of RA. Principal among these are the fact that until relatively recently, treatment has been considered lifelong for the majority of patients, imposing greater risk of toxicity and, as the immune system senesces, increased risk of infection or lymphoproliferative disease. There is little doubt that early treatment with tight control offers the best outcomes, and evaluation of synthetic and biological DMARDs in at-risk subjects to establish whether they can delay or even prevent development of clinically apparent disease could be transformative. A better appreciation of the profile of RA-specific autoantigens has prompted early-phase studies to establish the safety and tolerability of peptide immunotherapy delivered by autologous DCs. From an immunological perspective, there remains a pressing need to develop immunological tools or immune biomarkers that can redefine disease subsets and measure effector and regulatory cell subsets; progress has been made with synovial pathotypes. While such tools may provide better insights into disease pathogenesis, they can also be adapted to monitor the impact of therapeutic intervention, whether this turns out to be cell-based therapy or the application of novel immune modulators. Similar approaches should be used to identify those $ACPA^+$ arthralgia patients who have a genetic predisposition to RA and are at highest risk of developing the disease.

REFERENCES

1. Thomas R, Cope AP. Pathogenesis of Rheumatoid Arthritis. In: Watts R, ed. *Oxford Textbook of Rheumatology*. : Oxford University Press; 2016:839–848.
2. Frisell T, Saevarsdottir S, Askling J. Family history of rheumatoid arthritis: an old concept with new developments. *Nat Rev Rheumatol*. 2016;12:335.
3. van der Woude D, Houwing-Duistermaat JJ, Toes RE, et al. Quantitative heritability of anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis. *Arthritis Rheum*. 2009;60:916.
4. Karlson EW, Lee IM, Cook NR, et al. A retrospective cohort study of cigarette smoking and risk of rheumatoid arthritis in female health professionals. *Arthritis Rheum*. 1999;42:910.
5. Padyukov L, Silva C, Stolt P, et al. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum*. 2004;50:3085.
6. Lundberg K, Wegner N, Yucel-Lindberg T, et al. Periodontitis in RA—the citrullinated enolase connection. *Nat Rev Rheumatol*. 2010;6:727.
7. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med*. 2015;21:895.
8. Wells PM, Adebayo AS, Bowyer RCE et al. The gut microbiota and genetic risk for rheumatoid arthritis in the absence of disease: a TwinsUK association study. Lancet Rheumatology (in press).
9. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum*. 1987;30:1205.
10. Raychaudhuri S, Sandor C, Stahl EA, et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat Genet*. 2012;44:291.
11. Scally SW, Petersen J, Law SC, et al. A molecular basis for the association of the *HLA-DRB1* locus, citrullination and rheumatoid arthritis. *J Exp Med*. 2013;210:2569.
12. Eyre S, Bowes J, Diogo D, et al. High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat Genet*. 2012;44:1336.
13. Burn GL, Cornish GH, Potrzebowska K, et al. Super-resolution imaging of the cytoplasmic phosphatase PTPN22 links integrin-mediated adhesion with autoimmunity. *Sci Signaling*. 2016;9:ra99.
14. Manzo A, Bombardieri M, Humby F, et al. Secondary and ectopic lymphoid tissue responses in rheumatoid arthritis: from inflammation to autoimmunity and tissue damage/remodeling. *Immunol Rev*. 2010;233:267.
15. Lewis MJ, Barnes MR, Blighe K, et al. Molecular Portraits of Early Rheumatoid Arthritis Identify Clinical and Treatment Response Phenotypes. *Cell Rep*. 2019;28:2455–2470.
16. Mizoguchi F, Slowikowski K, Wei K, et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat Commun*. 2018;9:789.
17. Croft AP, Campos J, Jansen K, et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature*. 2019;570:246–251.
18. Culemann S, Grüneboom A, Nicolás-Ávila JÁ, et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. *Nature*. 2019;572:670–675.
19. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol*. 2007;7:429.
20. van Venrooij WJ, Vossenaar ER, Zendman AJ. Anti-CCP antibodies: the new rheumatoid factor in the serology of rheumatoid arthritis. *Autoimmun Rev*. 2004;3(Suppl. 1):S17.
21. Klareskog L, Rönnelid J, Lundberg K, et al. Immunity to citrullinated proteins in rheumatoid arthritis. *Annu Rev Immunol*. 2008;26:651.
22. Scherer HU, Huizinga TWJ, Krönke G, et al. The B cell response to citrullinated antigens in the development of rheumatoid arthritis. *Nat Rev Rheumatol*. 2018;14:157–169.
23. Rao DA, Gurish MF, Marshall JL, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature*. 2017;542:110–114.
24. Walter GJ, Fleskens V, Frederiksen KS, et al. Phenotypic, functional, and gene expression profiling of peripheral CD45RA+ and CD45RO+ CD4+CD25+CD127(low) Treg cells in patients with chronic rheumatoid arthritis. *Arthritis Rheumatol*. 2016;68:103.
25. Calabrese LH, Calabrese C, Cappelli LC. Rheumatic immune-related adverse events from cancer immunotherapy. *Nat Rev Rheumatol*. 2018;14:569–579.
26. Harre U, Schett G. Cellular and molecular pathways of structural damage in rheumatoid arthritis. *Semin Immunopathol*. 2017;39:355–363.
27. van Steenbergen HW, Aletaha D, Beaart-van de Voorde LJ, et al. EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis. *Ann Rheum Dis*. 2017;76:491–496.
28. Bos WH, Wolbink GJ, Boers M, et al. Arthritis development in patients with arthralgia is strongly associated with anti-citrullinated protein antibody status: a prospective cohort study. *Ann Rheum Dis*. 2010;69:490.
29. Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. *Arthritis Rheum*. 2005;52:3381.
30. Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu Rev Immunol*. 2001;19:163.
31. Kishimoto T. Interleukin-6: from basic science to medicine—40 years in immunology. *Annu Rev Immunol*. 2005;23:1.
32. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat Rev Drug Discov*. 2017;16:843–862.
33. Kremer JM, Westhovens R, Leon M, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N Engl J Med*. 2003;349:1907.
34. Edwards JC, Cambridge G. B-cell targeting in rheumatoid arthritis and other autoimmune diseases. *Nat Rev Immunol*. 2006;6:394.