HHS Public Access

Author manuscript

World J Transl Med. Author manuscript; available in PMC 2016 March 31.

Published in final edited form as:

World J Transl Med. 2015 December ; 4(3): 60–68. doi:10.5528/wjtm.v4.i3.60.

Contributions of neutrophils to the adaptive immune response in autoimmune disease

Kathryn M Pietrosimone and

Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

Peng Liu

Department of Medicine and Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

Abstract

Neutrophils are granulocytic cytotoxic leukocytes of the innate immune system that activate during acute inflammation. Neutrophils can also persist beyond the acute phase of inflammation to impact the adaptive immune response during chronic inflammation. In the context of the autoimmune disease, neutrophils modulating T and B cell functions by producing cytokines and chemokines, forming neutrophil extracellular traps, and acting as or priming antigen presentation cells. Thus, neutrophils are actively involved in chronic inflammation and tissue damage in autoimmune disease. Using rheumatoid arthritis as an example, this review focuses on functions of neutrophils in adaptive immunity and the therapeutic potential of these cells in the treatment of autoimmune disease and chronic inflammation.

Keywords

Neutrophils; Chronic inflammation; Autoimmune disease; Rheumatoid arthritis; Collagen-induced arthritis

INTRODUCTION

Neutrophils are polymorphonuclear granulocytes comprised of enzyme-containing granules. Neutrophils generate from the bone marrow and account for 50%–70% of circulating leukocytes in humans and 10%–25% in mice[1,2]. Under acute inflammation, particularly as a result of bacterial infection, neutrophils are the first leukocytes to respond, migrate to the site of inflammation, and kill microorganisms through phagocytosis, degranulation and

Correspondence to: Peng Liu, MD, PhD, Department of Medicine and Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, 3300 Thurston Building, CB#7280, Chapel Hill, NC 27599, United States. liupz@med.unc.edu, Telephone: +1−919−9660570, Fax: +1−919−9669269.

Author contributions: Both authors contributed to this paper.

Conflict-of-interest statement: The authors declare no conflict of interest.

generation of neutrophil extracellular traps (NETs)[3–5]. These cells have long been thought of as short-lived cells of the innate immune response.

However, recent research evidence has demonstrated that neutrophils persist beyond acute inflammation to initiate and perpetuate chronic inflammation. The onset of inflammation increases the lifespan of neutrophils in circulation, anywhere from 12 h to several days[6]. Pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ, inhibit neutrophil apoptosis[7]. Neutrophils also change phenotypes as inflammation persists. For instance, Neutrophils isolated from various inflammatory states show altered cell densities in gradient centrifugation[8,9]. The expression of granulocyte marker CD66b in neutrophils increases in patients with rheumatoid arthritis (RA), and decreases with the treatment by anti-inflammatory glucocorticoids, suggesting neutrophils may alter functions during chronic inflammation[10]. Glucocorticoids, a class of drugs often prescribed to patients with autoimmune disease, also inhibit apoptosis of neutrophils and increase the neutrophilic production of reactive oxygen species (ROS)[11].

Tissue-specific autoimmune disease such as RA, multiple sclerosis, and type I diabetes are generated from unknown etiology and impact the quality of life of patients through sustained chronic inflammation driven by innate and adaptive immune responses. Neutrophils bridge the innate and adaptive immune response in autoimmune disease. This review focuses on the functional heterogeneity of neutrophils in autoimmune disease and the contribution of these roles to chronic inflammation, in the context of RA.

RA

RA is an autoimmune disease, in which the body generates antibodies against its own tissues. RA is characterized by tissue-specific autoimmune-mediated chronic inflammation that affects multiple joints and results in destruction of cartilage and bone loss[12]. Risk factors of RA are multi-dimensional and include genetic defects, infections, and environmental influences[12].

Population studies of RA estimate that genetics account for about 50% of RA disease susceptibility[13–15]. The most consistent predictor of susceptibility to RA is the link between specific major histocompatibility (MHC) II-associated alleles of the *DRB1* gene that encode human leukocyte antigen (HLA)-DR4 and the onset of RA[16]. All of the susceptible alleles encode a conserved amino acid sequences on MHC II[17]. RA patients with these susceptible HLA-DR4 alleles and subsequent expression of the conserved amino acid sequence on MHC II, develop more autoantibodies associated with RA-related joint breakdown than patients without these alleles[18]. Activated antigen-presentation cells (APCs), including dendritic cells and macrophages, upregulate MHC II surface molecule expression, which activates antigen-specific T cells and B cells and initiates the adaptive immune responses in autoimmune disease.

Bacterial infections may trigger the onset of RA. A population-based study in Sweden determined that 45% of inflammatory arthritis patients had an infection prior to the onset of early arthritis[19]. In Chlamydia-induced reactive arthritis, the microbe primes neutrophils through toll-like receptor signaling, which activates the cell to clear the infection. However,

activated neutrophils can also infiltrate the joint and cause chronic inflammation[20]. Neutrophils may employ the same mechanisms in the onset of RA after an infection. Although many microbial factors have been found in synovial fluid of RA patients, there is no clear agreement that these microbial factors are the causative agents of RA[21–23].

Environmental factors, such as cigarette smoke, make provoke the development of RA in genetically susceptible populations[24,25]. Smokers with the HLA-DR shared epitope allele were 3 times more likely to test positive for rheumatoid factor (RF) than non-smokers with HLA-DR shared epitope allele[25]. Smoking increases the activation and migration of neutrophils[26,27]. Dysregulated neutrophils as a result of cigarette smoking generate a systemic inflammatory environment that is associated with autoimmune disease, such as RA and systemic lupus erythematosus[28,29].

Neutrophils account for the majority of inflammatory cells in the synovia of human RA patients, and the joints of collagen-induced arthritis (CIA) mice, a murine model of RA[30,31]. In both humans and mice, neutrophils accumulate at the pannus-cartilage interface, where much the destruction to both bone and cartilage takes places[32–34]. Thus, an in-depth understanding of neutrophil function in arthritis is vital to the prevention and treatment of this disease.

Neutrophil identification

The surface marker granulocyte receptor-1 (Gr-1) was previously used to identify neutrophil populations in murine models[35], as it was thought only mature granulocytes express Gr-1. Later researchers found that Gr-1 antibodies cross-react and bind two Ly-6 family member proteins, Ly6G and Ly6C[36,37]. While neutrophils express Ly6G, monocytes, memory T cells, and some dendritic cell subsets express Ly6C[36,38–40]. Studies utilize anti-Gr-1 antibodies to deplete neutrophils and attribute the presence of neutrophils to the ability of cancers cells to acquire metastatic phenotypes[41,42]. Although neutrophils make up the majority of Gr-1^+ cells in these studies, the increased metastasis after administration of anti-GR-1 antibodies cannot be attributed solely to neutrophil depletion, as $Ly6C^+$ monocyte are also depleted. In CIA, the depletion of neutrophils with anti-GR-1 antibody concluded that neutrophils were necessary for the onset and maintenance of disease, as this treatment prevented the onset of disease and ameliorated established disease[31]. Macrophages express Gr-1 and also infiltrate the CIA joint, so this method fails to delineate the roles of neutrophils from macrophages in CIA. Currently, studies utilize antibody clone IA8 to specifically bind Ly6G and deplete neutrophils without effect on other leukocyte populations[43].

In general, current murine studies identify neutrophils as $CD11b^+Ly6G^+Ly6C^+$ cells, and monocytes as $CD11b^+Ly6G^-Ly6C^+$ cells. Human neutrophils are defined as $CD14^{lo/neg}CD15^+CD16^{hi}CD33^+CD11b^+CD15^+CD66b^+$, and human monocytes are defined as $CD14^+CD16^+HLA^-$ DR^+CD66^- cells[44].

Neutrophil effect on T cells

Neutrophils, unlike dendritic cells and macrophages, are not defined as APCs. APCs express antigen to T cells *via* MHC II molecules and stimulates T cell activation with the aid of co-

stimulatory molecules CD80 and CD86. In autoimmunity, autoantigen is presented and antigen-activated T cells quickly expand and migrate to the site of inflammation and induce tissue inflammation through the production of pro-inflammatory cytokines[45]. In genetically susceptible individuals, the conserved amino acid sequence on MHC II may manipulate the antigen presentation process and cause activation of autoreactive T cells[46]. In the absence of autoimmune disease, neutrophils isolated from the peripheral blood of healthy controls produce MHC II mRNA, but do not express the cell surface molecule[47]. Exposure of these healthy neutrophils to synovial fluid from an RA patient elicits surface expression of both MHC II and costimulatory molecules[48]. Contact with T cells induces neutrophilic expression of MHC II and costimulatory molecules[48]. Since T cells are abundant in the RA joint, neutrophils continually express MHC II and costimulatory molecules and act as APCs. This further activates T cells and forms a vicious feedback loop that promotes chronic inflammation and tissue damage in the joints[48–50]. The continuous activation of T cells in the joint advances joint destruction through the production of pro-inflammatory cytokines and activation of autoreactive B cells[51,52].

Recently, studies have linked neutrophil functions to Th17 cells. Th17 cells, which produce IL-17, are potent proinflammatory mediators and have been implicated in the pathogenesis of autoimmune disease[53–56]. IL-17 can induce tissue inflammation by stimulating the recruitment of neutrophils. In the RA joint, IL-17 activates fibro-blast-like synoviocytes (FLS), macrophages, and osteoblasts[53–58]. FLS activation produces the potent neutrophil chemoattractant IL-8[59,60]. Activation of macrophages produces TNF-α[61], and the combination of Il-17 and TNF-α in the joint stimulates synovial endothelial cells to produce more neutrophil chemoattractants[62,63].

Reciprocally, neutrophils help sustain Th17 cells in the joint through the secretion of Th17 chemokines CCL20 and CCL2[60]. Human neutrophils purified from the synovial fluid of RA patients express high levels of these chemokines[60]. Despite the presence of other chemokines in the joint, Th17 cells preferentially migrate toward CCL20[64]. Mice that lack the CCL2 receptor ($CCR2^{-/-}$) develop exacerbated CIA. Furthermore, Th17 and neutrophil populations expand in the lymph nodes and joints of these mice[65]. Therefore, CCL20 likely plays a more prominent role in Th17 migration than CCL2. The expansion of both neutrophils and Th17 cells in mice with exacerbated arthritis demonstrates the importance of both these cell types to the onset and maintenance of autoimmune arthritis. The reciprocal signaling between Th17 cells and neutrophils causes accumulation and activation of these cells, cultivating an inflammatory microenvironment in the joint[58,60,66].

Although reducing neutrophils in the joint is beneficial to limit sustained T cell activation, systemic neutropenia can cause infection. Some therapeutics intervene with proinflammatory events that upregulate neutrophils, instead of depleting neutrophil function. A phase II trial that utilizes the anti-IL-17 monoclonal antibody drug, secukinumab, improves symptoms in 46% of RA patients after 16 wk of treatment, and can safely maintain these improvements through week 52[67,68]. Infection rates of patients on these drugs were 31.9% through week 52. Most infections were mild, but, interestingly, were not associated with neutropenia[68]. Simultaneously targeting neutrophils may improve IL-17 treatments, as activation of Th17 cells would decrease in addition to just decreasing the IL-17 effector

molecule. However, as with many autoimmune therapies, this may leave the patient susceptible to infection.

Neutrophil effect on B cells

One of the major features of autoimmune disease is the presence of autoantibodies in circulation. In RA patients, various autoantibodies against cartilage components, chaperones, enzymes, nuclear proteins, and citrullinated proteins have been identified. However, the clinical significances and pathogenic roles of these antibodies are largely unknown, except for RF, anti-citrullinated protein antibodies (ACPA), and anti-collagen antibodies, which are all associated with joint inflammation[69–72]. Neutrophils stimulate the activation, proliferation, differentiation, and antibody-production of B cells through the production of the B cell stimulating factor BAFF (also known as BLyS)[73–75]. Peripheral blood neutrophils from both RA patients and healthy control patients express BAFF as a membrane bound molecule[76]. However, TNF-α in the RA joint releases surface-bound BAFF from neutrophils and increases the concentration of soluble BAFF[76]. High levels of soluble BAFF in the serum of RA patients correlates with high concentrations of autoantibodies[77].

Neutrophils in the joint act on B cells similarly to splenic marginal zone neutrophils, which induce antibody production and immunoglobulin class switching through the production of the B cell stimulants BAFF, APRIL, and IL-21[78]. These stimulants drive the formation of splenic germinal centers that support the proliferation and differentiation of B cells. The blockage of BAFF decreases the size and disorganizes splenic germinal centers[79,80]. Structures similar to splenic germinal centers develop in the synovia of some RA patients[81]. A therapy that reduces soluble BAFF released from neutrophils may prevent synovial germinal center formation and reduce autoantibody production[82]. Thus, soluble BAFF from neutrophils plays a critical role in facilitating an environment, both systemically and locally, that activates B cells and perpetuates autoantibody formation.

The release of ROS by neutrophils[83,84] generates advanced glycation end-products (AGE) through oxidant-induced alteration of the structures of lipids, DNA, and proteins[83–86]. High levels of AGE in the sera of RA patients correlates with a high disease severity and high levels of inflammation markers[87]. B cells recognize ROS-modified structures as foreign molecules and produce autoantibodies against these structures[88,89]. ROS modifies type II collagen, the main structural component in human articular cartilage and induces the production of autoantibodies to ROS-modified collagen[90–94]. In an *in vitro* study, serum from RA patients could only bind type II collagen after exposure to ROS produced by neutrophils[95].

Autoantibodies form immune complexes in the joint, which induce neutrophil infiltration and activation through complement-mediated pathways[96,97]. B cells and neutrophils, therefore, work in concert to maintain inflammation in the RA joint.

Treatment with rituximab, a monoclonal anti-CD20 antibody that depletes B cells, decreases the severity of RA most efficiently in RA patients with high levels of autoantibodies[98,99]. Some patients experience late-onset neutropenia up to 12 mo following rituximab

treatment[99]. The exact mechanism that causes late-onset neutropenia is not known. One popular hypothesis suggests B cells compete with neutrophils for resources in the developmental niche of the bone marrow as the B cells repopulate after rituximab treatment[98].

Neutrophils and NET formation

Neutrophils form NETs through a unique model of cell death known as NETosis[100]. The formation of NETs requires activated neutrophils to lose integrity of intracellular membranes prior to that of the plasma membrane[101]. In the first steps of NET formation, granules containing cytotoxic antimicrobial proteins decay, and chromatin condenses as the nuclear membrane collapses. The plasma membrane then invaginates, ruptures, and releases NETs comprised of intracellular antimicrobial contents into the extracellular space[102]. In the context of infection, the antimicrobial proteins trap and kill infiltrating microbes in the extracellular space[100,103].

NETs release citrullinated histones and proteins into the extracellular space[104,105]. Citrullination of a protein or histone is a post-translational modification (PTM) that converts arginine residues to citrulline[104,105]. This process changes the structure and antigenicity of proteins and histones, as the adaptive immune response can recognize PTM as non-self[106]. In RA, ACPA are of particular interest as an increase in ACPA correlate with an increased disease severity[107,108]. An increased propensity for neutrophils to die *via* NETosis correlates with increased levels of ACPA in the serum of RA patients[105], which suggests NETs are a major source of autoantigen in RA. In fact, proteins extracted NET-induced peripheral blood neutrophils react with sera from RA patients. Sera from RA patients react specifically with citrullinated histone H4 [109].

The discovery of a conserved citrullinated antigen associated with the onset of RA opens up a novel avenue of therapeutic intervention. The enzyme PAD4 controls the citrullination of histone H4[109]. Interruption of PAD4 function could decrease citrullination of histone H4 and subsequent autoantibody production that is crucial to the development of RA.

NETosis also stimulates FLS to produce the proinflammatory cytokines IL-6, IL-8, and the Th17-associated chemokine CCL20[105]. Production of IL-6 and IL-8 aid in the polarization of CD4+ T cells to Th17 phenotypes, while CCL20 traffics Th17 cells to the of inflammation. Thus, NET stimulation of FLS shapes a microenvironment favorable to sustained inflammation associated with Th17 cells.

Neutrophils as myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells derived from the bone marrow under pathologic conditions that suppress T cell functions[110]. Murine MDSCs are divided into two subsets based on surface expression of Ly6G and Ly6C; the $Ly6G^{+}Ly6C^{+}CD11b^{+}$ granulocytic-MDSC (G-MDSC) subset and the $Ly6G\text{-}Ly6C^{+}CD11b^{+}$ monocytic-MDSC (M-MDSC) subset[111]. The counterparts of M-MDSC subset in humans have been identified as $CD11b^{+}CD14^{+}CD15^{-}HLA^{-}DR^{-}Lin^{-}$, while human G-MDSC subset are identified as $CD11b^{+}CD15^{+}CD14^{lo}HLA^{-}DR^{-}$ Lin^{-}[111] (Table 1). M-MDSCs are immature monocytes that suppress T cell functions through the

secretion of inducible nitric oxide synthase (iNOS)[112–114]. G-MDSCs are neutrophil-like cells that inhibit T cell function through the production of arginase-1[114].

First described in murine tumor models, MDSCs accumulate in the lymphoid tissues of tumor-bearing mice[115] and significantly increase in the circulation of cancer patients compared to healthy controls[116–118]. MDSCs promote tumor growth by inhibiting T-cell mediated immune surveillance and cytotoxic effects on tumor cells[119]. Recently, evidence indicates MDSCs also contribute to the regulation of autoimmune disease by inhibiting $CD4^+$ T cell proliferation and differentiation[120,121].

Neutrophil-like G-MDSCs are believed to be immunosuppressive in autoimmunity. In the experimental autoimmune encephalomyelitis (EAE) murine model of multiple sclerosis, G-MDSCs express high levels of programmed cell death 1 ligand 1 (PD-L1), a costimulatory molecule that negatively regulates T cell proliferation. G-MDSCs inhibit autoantigen-priming of Th1 and Th17 cells in a PD-L1-dependent manner[122]. In CIA, MDSCs that include mostly neutrophil-like G-MDSCs suppress both T cell proliferation and $CD4^+$ T cell differentiation into Th17 cells, mainly through the production of arginase-1[123]. The depletion of MDSCs increases inflammation and disease severity, while the subsequent adoptive transfer of MDSC ameliorates arthritis[123]. Furthermore, based on our published[124] and unpublished data, M-MDSCs as well as G-MDSCs not only suppress T cell functions, but also inhibit B cell proliferation in the context of CIA.

The use of $CD11b^+Gr\text{-}1^+$ cells in most functional MDSC studies makes it difficult to confirm G-MDSCs immunosuppressive effects in autoimmune arthritis Additionally, utilization of Ly6G to recognize G-MDSCs cannot differentiate G-MDSCs from neutrophils. Similar issues exist in human MDSCs, as mature human neutrophils express many of the same surface receptors as G-MDSCs and cannot be distinguished without functional analysis[125].

Further studies comparing G-MDSC phenotypes *vs* neutrophil phenotypes are needed to determine the effect of these cells on the adaptive immune response. Current evidence indicates G-MDSCs and neutrophils act in opposing manners on the adaptive immune response[125]. Uncovering mechanisms that lead to the plasticity of G-MDSCs and neutrophils in autoimmune disease could lead to cell-based therapies that convert pro-inflammatory cells to immunosuppressive cells.

Neutrophils and potential therapeutics

As one of major contributing factors in the establishment of chronic inflammation in RA, neutrophils serve as potential therapeutic targets. Some current therapies for RA interfere with the functions of neutrophils. For example, anti-TNF-α therapies reduce IL-33 receptor expression on neutrophils and subsequently decrease neutrophil migration. Neutrophils from RA patients treated with anti-TNF-α therapies do not respond to IL-33-mediated chemotaxis[126]. Impaired chemotaxis of neutrophils may lead to a decrease in inflammation and disease severity. The wide array of effector proteins produced by neutrophils, such as BAFF, could also become therapeutic targets for RA.

NETs may serve as a novel therapeutic target for RA and other NET-associated autoimmune diseases. In addition to targeting enzymes associated with PTM, the use of DNAse to breakdown the extracellular DNA and histones associated with NETs has been suggested as a potential therapeutic in NET-associated diseases[127]. The study of NETs in RA may also reveal currently unknown citrullinated proteins that contribute to RA pathogenesis and could serve as therapeutic targets.

In CIA, the adoptive transfer of MDSCs decrease T and B cell proliferation and decreases the severity of arthritis[123,124]. The discovery of a mechanism that induces the G-MDSC phenotype from neutrophil-like cells could be crucial for the therapy of autoimmune disease. Manipulation of this mechanism will drive neutrophil-like cells toward an immunosuppressive MDSC phenotype that impedes the super-active adaptive immune response, and thereby reduces chronic inflammation in autoimmunity. An in-depth understanding of the contributions of the joint microenvironment to the various neutrophil phenotypes, and subsequent neutrophil functions, may aid in the development of neutrophil-based RA therapies.

Similar to many autoimmune therapies, inhibition of neutrophil functions leaves the patients susceptible to infection. For this reason, the inhibition of specific enzymes, such as PAD4, may be a promising therapeutic intervention. If PAD4 can be neutralized, this could curb autoantibody production without completely diminishing the antimicrobial function of neutrophils.

CONCLUSION

Neutrophils are involved in the onset and progression of RA in a complex capacity. Neutrophils engage in several reciprocal signaling events with both B and T cells, which promote a microenvironment conducive to sustained inflammation. The formation of NETs increases the production of ROS and ACPA, which are hallmarks of RA. Neutrophils can act as pro-inflammatory cells influencing chemotaxis and immune cells signaling, but can also have a MDSC phenotype that suppresses the immune response. Since neutrophils affect many aspects of the adaptive immune response and drive chronic inflammation, the disruption of the signals between neutrophils and the adaptive immune response can serve as therapeutic targets for RA.

Acknowledgments

Supported by The National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health (NIAMS−NIH), No. 1R01AR063132.

References

1. Doeing DC, Borowicz JL, Crockett ET. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. BMC Clin Pathol. 2003; 3:3.10.1186/1472-6890-3-3 [PubMed: 12971830]
2. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004; 172:2731–2738. [PubMed: 14978070]

3. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013; 13:159–175.10.1038/nri3399 [PubMed: 23435331]
4. Borregaard N. Neutrophils, from marrow to microbes. Immunity. 2010; 33:657–670.10.1016/j.immuni.2010.11.011 [PubMed: 21094463]
5. Häger M, Cowland JB, Borregaard N. Neutrophil granules in health and disease. J Intern Med. 2010; 268:25–34.10.1111/j.1365-2796.2010.02237.x [PubMed: 20497300]
6. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER. Neutrophil kinetics in health and disease. Trends Immunol. 2010; 31:318–324.10.1016/j.it.2010.05.006 [PubMed: 20620114]
7. Keel M, Ungethüm U, Steckholzer U, Niederer E, Hartung T, Trentz O, Ertel W. Interleukin-10 counterregulates proinflammatory cytokine-induced inhibition of neutrophil apoptosis during severe sepsis. Blood. 1997; 90:3356–3363. [PubMed: 9345017]
8. Carmona-Rivera C, Kaplan MJ. Low-density granulocytes: a distinct class of neutrophils in systemic autoimmunity. Semin Immunopathol. 2013; 35:455–463.10.1007/s00281-013-0375-7 [PubMed: 23553215]
9. Denny MF, Yalavarthi S, Zhao W, Thacker SG, Anderson M, Sandy AR, McCune WJ, Kaplan MJ. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. J Immunol. 2010; 184:3284–3297.10.4049/jimmunol.0902199 [PubMed: 20164424]
10. Torsteinsdóttir I, Arvidson NG, Hällgren R, Håkansson L. Enhanced expression of integrins and CD66b on peripheral blood neutrophils and eosinophils in patients with rheumatoid arthritis, and the effect of glucocorticoids. Scand J Immunol. 1999; 50:433–439. [PubMed: 10520185]
11. Cox G. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. J Immunol. 1995; 154:4719–4725. [PubMed: 7722324]
12. Silman, AJ. Rheumatoid Arthritis. In: Silman, AJ.; Hochberg, MC., editors. Epidemiology of the rheumatid diseases. 2. Oxford: Oxford University Press; 2001. p. 31-71.
13. Bax M, van Heemst J, Huizinga TW, Toes RE. Genetics of rheumatoid arthritis: what have we learned? Immunogenetics. 2011; 63:459–466.10.1007/s00251-011-0528-6 [PubMed: 21556860]
14. Carty SM, Snowden N, Silman AJ. Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? J Rheumatol. 2003; 30:425–429. [PubMed: 12610794]
15. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, Silman AJ. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum. 2000; 43:30–37.10.1002/1529-0131(200001)43 [PubMed: 10643697]
16. Reveille JD. Genetic studies in the rheumatic diseases: present status and implications for the future. J Rheumatol Suppl. 2005; 72:10–13. [PubMed: 15660456]
17. 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; 30:1205–1213. [PubMed: 2446635]
18. Holoshitz J. The rheumatoid arthritis HLA-DRB1 shared epitope. Curr Opin Rheumatol. 2010; 22:293–298.10.1097/BOR.0b013e328336ba63 [PubMed: 20061955]
19. Söderlin MK, Kautiainen H, Puolakkainen M, Hedman K, Söderlund-Venermo M, Skogh T, Leirisalo-Repo M. Infections preceding early arthritis in southern Sweden: a prospective population-based study. J Rheumatol. 2003; 30:459–464. [PubMed: 12610801]
20. Zhang X, Glogauer M, Zhu F, Kim TH, Chiu B, Inman RD. Innate immunity and arthritis: neutrophil Rac and toll-like receptor 4 expression define outcomes in infection-triggered arthritis. Arthritis Rheum. 2005; 52:1297–1304.10.1002/art.20984 [PubMed: 15818670]
21. Bardoel BW, Kenny EF, Sollberger G, Zychlinsky A. The balancing act of neutrophils. Cell Host Microbe. 2014; 15:526–536.10.1016/j.chom.2014.04.011 [PubMed: 24832448]
22. Témoin S, Chakaki A, Askari A, El-Halaby A, Fitzgerald S, Marcus RE, Han YW, Bissada NF. Identification of oral bacterial DNA in synovial fluid of patients with arthritis with native and failed prosthetic joints. J Clin Rheumatol. 2012; 18:117–121.10.1097/RHU.0b013e3182500c95 [PubMed: 22426587]

23. Ataee RA, Ataee MH, Alishiri GH, Esmaeili D. Staphylococcal enterotoxin C in synovial fluid of patients with rheumatoid arthritis. Iran Red Crescent Med J. 2014; 16:e16075.10.5812/ircmj.16075 [PubMed: 25558381]
24. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, Alfredsson L. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Ann Rheum Dis. 2003; 62:835–841. [PubMed: 12922955]
25. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum. 2004; 50:3085–3092.10.1002/art.20553 [PubMed: 15476204]
26. Blidberg K, Palmberg L, Dahlén B, Lantz AS, Larsson K. Increased neutrophil migration in smokers with or without chronic obstructive pulmonary disease. Respirology. 2012; 17:854–860.10.1111/j.1440-1843.2012.02181.x [PubMed: 22509802]
27. Pitzer JE, Del Zoppo GJ, Schmid-Schönbein GW. Neutrophil activation in smokers. Biorheology. 1996; 33:45–58. [PubMed: 8869343]
28. Baka Z, Buzás E, Nagy G. Rheumatoid arthritis and smoking: putting the pieces together. Arthritis Res Ther. 2009; 11:238.10.1186/ar2751 [PubMed: 19678909]
29. Majka DS, Holers VM. Cigarette smoking and the risk of systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis. 2006; 65:561–563.10.1136/ard.2005.046052 [PubMed: 16611864]
30. Brand DD, Latham KA, Rosloniec EF. Collagen-induced arthritis. Nat Protoc. 2007; 2:1269–1275.10.1038/nprot.2007.173 [PubMed: 17546023]
31. Tanaka D, Kagari T, Doi H, Shimozato T. Essential role of neutrophils in anti-type II collagen antibody and lipopolysaccharide-induced arthritis. Immunology. 2006; 119:195–202.10.1111/j.1365-2567.2006.02424.x [PubMed: 16836650]
32. Mohr W, Westerhellweg H, Wessinghage D. Polymorphonuclear granulocytes in rheumatic tissue destruction. III. an electron microscopic study of PMNs at the pannus-cartilage junction in rheumatoid arthritis. Ann Rheum Dis. 1981; 40:396–399. [PubMed: 7259331]
33. Murphy G, Nagase H. Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? Nat Clin Pract Rheumatol. 2008; 4:128–135.10.1038/ncprheum0727 [PubMed: 18253109]
34. Wright HL, Moots RJ, Edwards SW. The multifactorial role of neutrophils in rheumatoid arthritis. Nat Rev Rheumatol. 2014; 10:593–601.10.1038/nrrheum.2014.80 [PubMed: 24914698]
35. Tepper RI, Coffman RL, Leder P. An eosinophil-dependent mechanism for the antitumor effect of interleukin-4. Science. 1992; 257:548–551. [PubMed: 1636093]
36. Rose S, Misharin A, Perlman H. A novel Ly6C/Ly6G-based strategy to analyze the mouse splenic myeloid compartment. Cytometry A. 2012; 81:343–350.10.1002/cyto.a.22012 [PubMed: 22213571]
37. Fleming TJ, Fleming ML, Malek TR. Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. J Immunol. 1993; 151:2399–2408. [PubMed: 8360469]
38. Goldrath AW, Bogatzki LY, Bevan MJ. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. J Exp Med. 2000; 192:557–564. [PubMed: 10952725]
39. Marshall HD, Chandele A, Jung YW, Meng H, Poholek AC, Parish IA, Rutishauser R, Cui W, Kleinstein SH, Craft J, Kaech SM. Differential expression of Ly6C and T-bet distinguish effector and memory Th1 CD4(+) cell properties during viral infection. Immunity. 2011; 35:633–646.10.1016/j.immuni.2011.08.016 [PubMed: 22018471]
40. Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, Vanhoutte L, Neyt K, Killeen N, Malissen B, Hammad H, Lambrecht BN. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. Immunity. 2013; 38:322–335.10.1016/j.immuni.2012.10.016 [PubMed: 23352232]

41. Spicer JD, McDonald B, Cools-Lartigue JJ, Chow SC, Giannias B, Kubes P, Ferri LE. Neutrophils promote liver metastasis via Mac-1-mediated interactions with circulating tumor cells. Cancer Res. 2012; 72:3919–3927.10.1158/0008-5472.CAN-11-2393 [PubMed: 22751466]

42. Tazawa H, Okada F, Kobayashi T, Tada M, Mori Y, Une Y, Sendo F, Kobayashi M, Hosokawa M. Infiltration of neutrophils is required for acquisition of metastatic phenotype of benign murine fibrosarcoma cells: implication of inflammation-associated carcinogenesis and tumor progression. Am J Pathol. 2003; 163:2221–2232.10.1016/S0002-9440(10)63580-8 [PubMed: 14633597]

43. Daley JM, Thomay AA, Connolly MD, Reichner JS, Albina JE. Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. J Leukoc Biol. 2008; 83:64–70.10.1189/Jlb.0407247 [PubMed: 17884993]

44. Abeles RD, McPhail MJ, Sowter D, Antoniades CG, Vergis N, Vijay GK, Xystrakis E, Khamri W, Shawcross DL, Ma Y, Wendon JA, Vergani D. CD14, CD16 and HLA-DR reliably identifies human monocytes and their subsets in the context of pathologically reduced HLA-DR expression by CD14(hi)/CD16(neg) monocytes: Expansion of CD14(hi)/CD16(pos) and contraction of CD14(lo)/CD16(pos) monocytes in acute liver failure. Cytometry A. 2012; 81:823–834.10.1002/cyto.a.22104 [PubMed: 22837127]

45. Murphy, K.; Travers, P.; Walport, M.; Janeway, C. Janeway's immunobiology. 8. New York: Garland Science; 2012.

46. Steiner G. Auto-antibodies and autoreactive T-cells in rheumatoid arthritis: pathogenetic players and diagnostic tools. Clin Rev Allergy Immunol. 2007; 32:23–36. [PubMed: 17426358]

47. Cross A, Bucknall RC, Cassatella MA, Edwards SW, Moots RJ. Synovial fluid neutrophils transcribe and express class II major histocompatibility complex molecules in rheumatoid arthritis. Arthritis Rheum. 2003; 48:2796–2806.10.1002/art.11253 [PubMed: 14558085]

48. Iking-Konert C, Ostendorf B, Sander O, Jost M, Wagner C, Joosten L, Schneider M, Hänsch GM. Transdifferentiation of polymorphonuclear neutrophils to dendritic-like cells at the site of inflammation in rheumatoid arthritis: evidence for activation by T cells. Ann Rheum Dis. 2005; 64:1436–1442.10.1136/ard.2004.034132 [PubMed: 15778239]

49. Radsak M, Iking-Konert C, Stegmaier S, Andrassy K, Hänsch GM. Polymorphonuclear neutrophils as accessory cells for T-cell activation: major histocompatibility complex class II restricted antigen-dependent induction of T-cell proliferation. Immunology. 2000; 101:521–530. [PubMed: 11122456]

50. Fanger NA, Liu C, Guyre PM, Wardwell K, O'Neil J, Guo TL, Christian TP, Mudzinski SP, Gosselin EJ. Activation of human T cells by major histocompatability complex class II expressing neutrophils: proliferation in the presence of superantigen, but not tetanus toxoid. Blood. 1997; 89:4128–4135. [PubMed: 9166855]

51. Cope AP. T cells in rheumatoid arthritis. Arthritis Res Ther. 2008; 10(Suppl 1):S1.10.1186/ar2412 [PubMed: 19007421]

52. Cope AP, Schulze-Koops H, Aringer M. The central role of T cells in rheumatoid arthritis. Clin Exp Rheumatol. 2007; 25:S4–11. [PubMed: 17977483]

53. Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol. 2008; 8:337–348.10.1038/nri2295 [PubMed: 18408735]

54. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005; 201:233–240.10.1084/jem.20041257 [PubMed: 15657292]

55. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol. 2008; 9:641–649.10.1038/ni.1610 [PubMed: 18454151]

56. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev. 2014; 13:668–677.10.1016/j.autrev.2013.12.004 [PubMed: 24418308]

57. Ferraccioli G, Zizzo G. The potential role of Th17 in mediating the transition from acute to chronic autoimmune inflammation: rheumatoid arthritis as a model. Discov Med. 2011; 11:413–424. [PubMed: 21616040]

58. Miossec P, Kolls JK. Targeting IL-17 and TH17 cells in chronic inflammation. Nat Rev Drug Discov. 2012; 11:763–776.10.1038/nrd3794 [PubMed: 23023676]

59. Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saeland S, Blanchard D, Gaillard C, Das Mahapatra B, Rouvier E, Golstein P, Banchereau J, Lebecque S. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med. 1996; 183:2593–2603. [PubMed: 8676080]
60. Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, Cosmi L, Lunardi C, Annunziato F, Romagnani S, Cassatella MA. Evidence for a cross-talk between human neutrophils and Th17 cells. Blood. 2010; 115:335–343.10.1182/blood-2009-04-216085 [PubMed: 19890092]
61. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, Mineau F, Pelletier JP. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol. 1998; 160:3513–3521. [PubMed: 9531313]
62. Griffin GK, Newton G, Tarrio ML, Bu DX, Maganto-Garcia E, Azcutia V, Alcaide P, Grabie N, Luscinskas FW, Croce KJ, Lichtman AH. IL-17 and TNF-α sustain neutrophil recruitment during inflammation through synergistic effects on endothelial activation. J Immunol. 2012; 188:6287–6299.10.4049/jimmunol.1200385 [PubMed: 22566565]
63. Middleton J, Americh L, Gayon R, Julien D, Aguilar L, Amalric F, Girard JP. Endothelial cell phenotypes in the rheumatoid synovium: activated, angiogenic, apoptotic and leaky. Arthritis Res Ther. 2004; 6:60–72.10.1186/ar1156 [PubMed: 15059266]
64. Hirota K, Yoshitomi H, Hashimoto M, Maeda S, Teradaira S, Sugimoto N, Yamaguchi T, Nomura T, Ito H, Nakamura T, Sakaguchi N, Sakaguchi S. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. J Exp Med. 2007; 204:2803–2812.10.1084/jem.20071397 [PubMed: 18025126]
65. Rampersad RR, Tarrant TK, Vallanat CT, Quintero-Matthews T, Weeks MF, Esserman DA, Clark J, Di Padova F, Patel DD, Fong AM, Liu P. Enhanced Th17-cell responses render CCR2-deficient mice more susceptible for autoimmune arthritis. PLoS One. 2011; 6:e25833.10.1371/journal.pone.0025833 [PubMed: 21991368]
66. Stamp LK, James MJ, Cleland LG. Interleukin-17: the missing link between T-cell accumulation and effector cell actions in rheumatoid arthritis? Immunol Cell Biol. 2004; 82:1–9.10.1111/j.1440-1711.2004.01212.x [PubMed: 14984588]
67. Kellner H. Targeting interleukin-17 in patients with active rheumatoid arthritis: rationale and clinical potential. Ther Adv Musculoskelet Dis. 2013; 5:141–152.10.1177/1759720X13485328 [PubMed: 23858337]
68. Genovese MC, Durez P, Richards HB, Supronik J, Dokoupilova E, Aelion JA, Lee SH, Codding CE, Kellner H, Ikawa T, Hugot S, Ligozio G, Mpofu S. One-year efficacy and safety results of secukinumab in patients with rheumatoid arthritis: phase II, dose-finding, double-blind, randomized, placebo-controlled study. J Rheumatol. 2014; 41:414–421.10.3899/jrheum.130637 [PubMed: 24429175]
69. Bugatti S, Vitolo B, Caporali R, Montecucco C, Manzo A. B cells in rheumatoid arthritis: from pathogenic players to disease biomarkers. Biomed Res Int. 2014; 2014:681678.10.1155/2014/681678 [PubMed: 24877127]
70. Hardy RR, Hayakawa K, Shimizu M, Yamasaki K, Kishimoto T. Rheumatoid factor secretion from human Leu-1+ B cells. Science. 1987; 236:81–83. [PubMed: 3105057]
71. Sakkas LI, Bogdanos DP, Katsiari C, Platsoucas CD. Anti-citrullinated peptides as autoantigens in rheumatoid arthritis-relevance to treatment. Autoimmun Rev. 2014; 13:1114–1120.10.1016/j.autrev.2014.08.012 [PubMed: 25182207]
72. Silverman GJ, Carson DA. Roles of B cells in rheumatoid arthritis. Arthritis Res Ther. 2003; 5(Suppl 4):S1–S6.10.1186/ar1010 [PubMed: 15180890]
73. Benson MJ, Dillon SR, Castigli E, Geha RS, Xu S, Lam KP, Noelle RJ. Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. J Immunol. 2008; 180:3655–3659. [PubMed: 18322170]
74. Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. Nat Rev Immunol. 2002; 2:465–475.10.1038/nri844 [PubMed: 12094221]
75. Rolink AG, Melchers F. BAFFled B cells survive and thrive: roles of BAFF in B-cell development. Curr Opin Immunol. 2002; 14:266–275. [PubMed: 11869903]

76. Assi LK, Wong SH, Ludwig A, Raza K, Gordon C, Salmon M, Lord JM, Scheel-Toellner D. Tumor necrosis factor alpha activates release of B lymphocyte stimulator by neutrophils infiltrating the rheumatoid joint. Arthritis Rheum. 2007; 56:1776–1786.10.1002/art.22697 [PubMed: 17530706]
77. Pers JO, Daridon C, Devauchelle V, Jousse S, Saraux A, Jamin C, Youinou P. BAFF overexpression is associated with autoantibody production in autoimmune diseases. Ann N Y Acad Sci. 2005; 1050:34–39.10.1196/annals.1313.004 [PubMed: 16014518]
78. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nat Rev Immunol. 2013; 13:118–132.10.1038/nri3383 [PubMed: 23348416]
79. Vora KA, Wang LC, Rao SP, Liu ZY, Majeau GR, Cutler AH, Hochman PS, Scott ML, Kalled SL. Cutting edge: germinal centers formed in the absence of B cell-activating factor belonging to the TNF family exhibit impaired maturation and function. J Immunol. 2003; 171:547–551. [PubMed: 12847217]
80. Rahman ZS, Rao SP, Kalled SL, Manser T. Normal induction but attenuated progression of germinal center responses in BAFF and BAFF-R signaling-deficient mice. J Exp Med. 2003; 198:1157–1169.10.1084/jem.20030495 [PubMed: 14557413]
81. Weyand CM, Goronzy JJ. Ectopic germinal center formation in rheumatoid synovitis. Ann N Y Acad Sci. 2003; 987:140–149. [PubMed: 12727633]
82. Fox DA, Gizinski A, Morgan R, Lundy SK. Cell-cell interactions in rheumatoid arthritis synovium. Rheum Dis Clin North Am. 2010; 36:311–323.10.1016/j.rdc.2010.02.004 [PubMed: 20510236]
83. Robinson J, Watson F, Bucknall RC, Edwards SW. Activation of neutrophil reactive-oxidant production by synovial fluid from patients with inflammatory joint disease. Soluble and insoluble immunoglobulin aggregates activate different pathways in primed and unprimed cells. Biochem J. 1992; 286(Pt 2):345–351. [PubMed: 1530567]
84. Robinson JJ, Watson F, Bucknall RC, Edwards SW. Stimulation of reactive oxidant production in neutrophils by soluble and insoluble immune complexes occurs via different receptors/signal transduction systems. FEMS Immunol Med Microbiol. 1994; 8:249–257. [PubMed: 8004062]
85. Drinda S, Franke S, Rüster M, Petrow P, Pullig O, Stein G, Hein G. Identification of the receptor for advanced glycation end products in synovial tissue of patients with rheumatoid arthritis. Rheumatol Int. 2005; 25:411–413.10.1007/s00296-004-0456-y [PubMed: 15045525]
86. Drinda S, Franke S, Canet CC, Petrow P, Bräuer R, Hüttich C, Stein G, Hein G. Identification of the advanced glycation end products N(epsilon)-carboxymethyllysine in the synovial tissue of patients with rheumatoid arthritis. Ann Rheum Dis. 2002; 61:488–492. [PubMed: 12006318]
87. Takahashi M, Suzuki M, Kushida K, Miyamoto S, Inoue T. Relationship between pentosidine levels in serum and urine and activity in rheumatoid arthritis. Br J Rheumatol. 1997; 36:637–642. [PubMed: 9236672]
88. Marcinkiewicz J, Biedroń R, Maresz K, Kwaśny-Krochin B, Bobek M, Kontny E, Maśliński W, Chain B. Oxidative modification of type II collagen differentially affects its arthritogenic and tolerogenic capacity in experimental arthritis. Arch Immunol Ther Exp (Warsz). 2004; 52:284–291. [PubMed: 15467493]
89. Strollo R, Ponchel F, Malmström V, Rizzo P, Bombardieri M, Wenham CY, Landy R, Perret D, Watt F, Corrigall VM, Winyard PG, Pozzilli P, Conaghan PG, Panayi GS, Klareskog L, Emery P, Nissim A. Autoantibodies to posttranslationally modified type II collagen as potential biomarkers for rheumatoid arthritis. Arthritis Rheum. 2013; 65:1702–1712.10.1002/Art.37964 [PubMed: 23575908]
90. Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. Sports Health. 2009; 1:461–468.10.1177/1941738109350438 [PubMed: 23015907]
91. McDevitt CA. Biochemistry of articular cartilage. Nature of proteoglycans and collagen of articular cartilage and their role in ageing and in osteoarthrosis. Ann Rheum Dis. 1973; 32:364–378. [PubMed: 4269430]
92. Wooley PH, Luthra HS, O'Duffy JD, Bunch TW, Moore SB, Stuart JM. Anti-type II collagen antibodies in rheumatoid arthritis. The influence of HLA phenotype. Tissue Antigens. 1984; 23:263–269. [PubMed: 6589808]

93. Menzel J, Steffen C, Kolarz G, Kojer M, Smolen J. Demonstration of anticollagen antibodies in rheumatoid arthritis synovial fluids by 14C-radioimmunoassay. Arthritis Rheum. 1978; 21:243–248. [PubMed: 637891]

94. Menzel J, Steffen C, Kolarz G, Eberal G, Frank O, Thumb N. Demonstration of antibodies to collagen and of collagen-anticollagen immune complexes in rheumatoid arthritis synovial fluids. Ann Rheum Dis. 1975; 35:446–450. [PubMed: 185972]

95. Nissim A, Winyard PG, Corrigall V, Fatah R, Perrett D, Panayi G, Chernajovsky Y. Generation of neoantigenic epitopes after posttranslational modification of type II collagen by factors present within the inflamed joint. Arthritis Rheum. 2005; 52:3829–3838.10.1002/art.21479 [PubMed: 16329077]

96. Iwanami K, Matsumoto I, Tanaka Y, Inoue A, Goto D, Ito S, Tsutsumi A, Sumida T. Arthritogenic T cell epitope in glucose-6-phosphate isomerase-induced arthritis. Arthritis Res Ther. 2008; 10:R130.10.1186/ar2545 [PubMed: 18992137]

97. Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, Takahashi K, Holers VM, Walport M, Gerard C, Ezekowitz A, Carroll MC, Brenner M, Weissleder R, Verbeek JS, Duchatelle V, Degott C, Benoist C, Mathis D. Arthritis critically dependent on innate immune system players. Immunity. 2002; 16:157–168. [PubMed: 11869678]

98. Ram R, Ben-Bassat I, Shpilberg O, Polliack A, Raanani P. The late adverse events of rituximab therapy--rare but there! Leuk Lymphoma. 2009; 50:1083–1095.10.1080/10428190902934944 [PubMed: 19399690]

99. Abdulkader R, Dharmapalaiah C, Rose G, Shand LM, Clunie GP, Watts RA. Late-onset neutropenia in patients with rheumatoid arthritis after treatment with rituximab. J Rheumatol. 2014; 41:858–861.10.3899/jrheum.130526 [PubMed: 24634201]

100. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. Science. 2004; 303:1532–1535.10.1126/science.1092385 [PubMed: 15001782]

101. Steinberg BE, Grinstein S. Unconventional roles of the NADPH oxidase: signaling, ion homeostasis, and cell death. Sci STKE. 2007:pe11.10.1126/stke.3792007pe11 [PubMed: 17392241]

102. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol. 2007; 176:231–241.10.1083/jcb.200606027 [PubMed: 17210947]

103. Remijsen Q, Kuijpers TW, Wirawan E, Lippens S, Vandenabeele P, Vanden Berghe T. Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. Cell Death Differ. 2011; 18:581–588.10.1038/cdd.2011.1 [PubMed: 21293492]

104. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med. 2010; 207:1853–1862.10.1084/Jem.20100239 [PubMed: 20733033]

105. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, Friday S, Li S, Patel RM, Subramanian V, Thompson P, Chen P, Fox DA, Pennathur S, Kaplan MJ. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci Transl Med. 2013; 5:178r.10.1126/scitranslmed.3005580

106. Anzilotti C, Pratesi F, Tommasi C, Migliorini P. Peptidylarginine deiminase 4 and citrullination in health and disease. Autoimmun Rev. 2010; 9:158–160.10.1016/j.autrev.2009.06.002 [PubMed: 19540364]

107. Aggarwal R, Liao K, Nair R, Ringold S, Costenbader KH. Anti-citrullinated peptide antibody assays and their role in the diagnosis of rheumatoid arthritis. Arthritis Rheum. 2009; 61:1472–1483.10.1002/art.24827 [PubMed: 19877103]

108. Kidd BA, Ho PP, Sharpe O, Zhao X, Tomooka BH, Kanter JL, Steinman L, Robinson WH. Epitope spreading to citrullinated antigens in mouse models of autoimmune arthritis and demyelination. Arthritis Res Ther. 2008; 10:R119.10.1186/ar2523 [PubMed: 18826638]

109. Pratesi F, Dioni I, Tommasi C, Alcaro MC, Paolini I, Barbetti F, Boscaro F, Panza F, Puxeddu I, Rovero P, Migliorini P. Antibodies from patients with rheumatoid arthritis target citrullinated

histone 4 contained in neutrophils extracellular traps. Ann Rheum Dis. 2014; 73:1414–1422.10.1136/annrheumdis-2012-202765 [PubMed: 23727635]

110. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009; 9:162–174.10.1038/nri2506 [PubMed: 19197294]
111. Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer. 2013; 13:739–752.10.1038/nrc3581 [PubMed: 24060865]
112. Jayaraman P, Parikh F, Lopez-Rivera E, Hailemichael Y, Clark A, Ma G, Cannan D, Ramacher M, Kato M, Overwijk WW, Chen SH, Umansky VY, Sikora AG. Tumor-expressed inducible nitric oxide synthase controls induction of functional myeloid-derived suppressor cells through modulation of vascular endothelial growth factor release. J Immunol. 2012; 188:5365–5376.10.4049/jimmunol.1103553 [PubMed: 22529296]
113. Kusmartsev SA, Li Y, Chen SH. Gr-1+ myeloid cells derived from tumor-bearing mice inhibit primary T cell activation induced through CD3/CD28 costimulation. J Immunol. 2000; 165:779–785. [PubMed: 10878351]
114. Sinha P, Clements VK, Ostrand-Rosenberg S. Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. Cancer Res. 2005; 65:11743–11751.10.1158/0008-5472.CAN-05-0045 [PubMed: 16357187]
115. Ezernitchi AV, Vaknin I, Cohen-Daniel L, Levy O, Manaster E, Halabi A, Pikarsky E, Shapira L, Baniyash M. TCR zeta down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. J Immunol. 2006; 177:4763–4772. [PubMed: 16982917]
116. Chi N, Tan Z, Ma K, Bao L, Yun Z. Increased circulating myeloid-derived suppressor cells correlate with cancer stages, interleukin-8 and −6 in prostate cancer. Int J Clin Exp Med. 2014; 7:3181–3192. [PubMed: 25419348]
117. Khaled YS, Ammori BJ, Elkord E. Increased levels of granulocytic myeloid-derived suppressor cells in peripheral blood and tumour tissue of pancreatic cancer patients. J Immunol Res. 2014; 2014:879897.10.1155/2014/879897 [PubMed: 24741628]
118. Zhang B, Wang Z, Wu L, Zhang M, Li W, Ding J, Zhu J, Wei H, Zhao K. Circulating and tumor-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. PLoS One. 2013; 8:e57114.10.1371/journal.pone.0057114 [PubMed: 23437326]
119. Haabeth OA, Lorvik KB, Hammarström C, Donaldson IM, Haraldsen G, Bogen B, Corthay A. Inflammation driven by tumour-specific Th1 cells protects against B-cell cancer. Nat Commun. 2011; 2:240.10.1038/ncomms1239 [PubMed: 21407206]
120. Cripps JG, Gorham JD. MDSC in autoimmunity. Int Immunopharmacol. 2011; 11:789–793.10.1016/j.intimp.2011.01.026 [PubMed: 21310255]
121. Crook KR, Liu P. Role of myeloid-derived suppressor cells in autoimmune disease. World J Immunol. 2014; 4:26–33.10.5411/wji.v4.i1.26 [PubMed: 25621222]
122. Ioannou M, Alissafi T, Lazaridis I, Deraos G, Matsoukas J, Gravanis A, Mastorodemos V, Plaitakis A, Sharpe A, Boumpas D, Verginis P. Crucial role of granulocytic myeloid-derived suppressor cells in the regulation of central nervous system autoimmune disease. J Immunol. 2012; 188:1136–1146.10.4049/jimmunol.1101816 [PubMed: 22210912]
123. Fujii W, Ashihara E, Hirai H, Nagahara H, Kajitani N, Fujioka K, Murakami K, Seno T, Yamamoto A, Ishino H, Kohno M, Maekawa T, Kawahito Y. Myeloid-derived suppressor cells play crucial roles in the regulation of mouse collagen-induced arthritis. J Immunol. 2013; 191:1073–1081.10.4049/jimmunol.1203535 [PubMed: 23804709]
124. Crook KR, Jin M, Weeks MF, Rampersad RR, Baldi RM, Glekas AS, Shen Y, Esserman DA, Little P, Schwartz TA, Liu P. Myeloid-derived suppressor cells regulate T cell and B cell responses during autoimmune disease. J Leukoc Biol. 2015; 97:573–582.10.1189/jlb.4A0314-139R [PubMed: 25583578]
125. Pillay J, Tak T, Kamp VM, Koenderman L. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. Cell Mol Life Sci. 2013; 70:3813–3827.10.1007/s00018-013-1286-4 [PubMed: 23423530]
126. Verri WA, Souto FO, Vieira SM, Almeida SC, Fukada SY, Xu D, Alves-Filho JC, Cunha TM, Guerrero AT, Mattos-Guimaraes RB, Oliveira FR, Teixeira MM, Silva JS, McInnes IB, Ferreira

SH, Louzada-Junior P, Liew FY, Cunha FQ. IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy. Ann Rheum Dis. 2010; 69:1697–1703.10.1136/ard.2009.122655 [PubMed: 20472598]

127. Diamantopoulos AP. Extracellular neutrophil traps: a novel therapeutic target in ANCA-associated vasculitis? Front Immunol. 2013; 4:24.10.3389/fimmu.2013.00024 [PubMed: 23382733]

Table 1

Surface markers used in identification of myeloid-derived suppressor cells

	Human	Mouse
MDSC	$CD11b^{+}$ $CD33^{+}$	$CD11b^{+}$ $Gr\text{-}1^{+}$
M-MDSC	$CD11b^{+}CD14^{+}CD15^{-}$ $HLA^{-}DR^{-}Lin^{-}$	$CD11b^{+}$ $Ly6C^{+}$ $Ly6G^{-}$
G-MDSC	$Cd11b^{+}CD15^{+}CD14^{lo}$ $HLA^{-}DR^{-}Lin^{-}$	$CD11b^{+}$ $Ly6G^{+}$ $Ly6C^{+}$
Progenitor neutrophil	$CD14^{lo/neg}$ $CD15^{+}$ $CD16^{lo/neg}$	
Mature neutrophil	$CD14^{lo/neg}$ $CD15^{+}$ $CD16^{hi}$ $CD33^{+}$ $CD11b^{+}$ $CD15^{+}$ $CD66b^{+}$	$CD11b^{+}$ $Ly6G^{+}$

MDSC: Myeloid-derived suppressor cell; M-MDSC: Monocytic-MDSC; G-MDSC: Granulocytic-MDSC.