

## **Macrophage heterogeneity in the context of rheumatic diseases**

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**Acknowledgments:** We thank M. Weiss for assistance with figure designs. IAU is supported by the Kennedy Institute Trustees' Research Fund and Arthritis Research UK. AM is supported by grants from Fondazione CARIPLO, the Italian Ministry of Health and European Commission (BTcure).

### **Abstract**

Macrophages are very important in the pathogenesis of rheumatoid arthritis (RA). The increase in the number of sublining macrophages in the synovium is an early hallmark of active rheumatic disease, and high numbers of macrophages are a prominent feature of inflammatory lesions. The degree of synovial macrophage infiltration correlates with the degree of joint erosion and their depletion from inflamed tissue has a profound therapeutic benefit. Research has now uncovered an unexpected level of heterogeneity of macrophage origin and function, and has emphasized the role of environmental factors in functional specialization. Although the heterogeneity of macrophages in RA has not been fully determined, preliminary results have contributed to understanding of the phenotype and ontogeny of synovial macrophages in mouse models of arthritis and to deciphering the properties of infiltrating monocyte-derived and tissue-resident macrophages. Elucidating the molecular mechanisms that drive polarization of macrophages towards inflammatory or protective phenotypes could lead to identification of signalling pathways that suggest future therapeutic strategies.

### **Introduction**

By far the best-studied form of arthritis at the cellular and molecular level is rheumatoid arthritis (RA). In addition to extensive research into RA, clinical trials have validated several of the principal molecules involved. Macrophages are very important in RA, as judged by many criteria, such as the augmented macrophage numbers during active disease and their diminution after effective treatment. Much less data exist concerning the roles of macrophages in rheumatic diseases other than RA. Macrophages are highly 'plastic' cells that can respond rapidly to many stimuli, making them potentially very important in disease pathogenesis. This Review discusses contemporary aspects of macrophage biology, along with the current

understanding of the pathways involved in macrophage heterogeneity, in both development and the response to environmental cues. Although macrophages are important in RA, the disease is not solely dependent on macrophages, but also on systemically activated myeloid and nonmyeloid cell types. However, a detailed analysis of the role of these cells is outside scope of this Review.

### **An historical perspective**

In 1967, the results were published of an examination of macrophages in synovial fluids from patients with a variety of inflammatory diseases<sup>1</sup>. The investigation was an assessment of the quantitative and morphological changes in these cells that could be diagnostically helpful, and the conclusion was that large, phagocytic cells were rare in fluids from adults with RA, so they had limited diagnostic significance. The advent of synovial needle biopsy and the increasing use of synovectomy and other surgical corrective procedures in the management of arthritis in the 1980s contributed to the availability of joint tissue for histopathological examination<sup>2</sup>. When biopsy specimens from synovial membranes of patients with RA were compared with normal synovial membranes, the diseased tissue contained greater numbers of large, HLA-DR-positive (macrophage-like cells)<sup>3</sup>. These cells made up almost the entire synovial lining, and their numbers were also dramatically increased in the sublining tissue<sup>3</sup>. This seminal work led to the hypothesis—which has been experimentally investigated and provided with further detail ever since its publication—that macrophages in the synovial membranes of patients with RA drive T-cell infiltration via antigen presentation. This action can trigger B-cell infiltration and production of immunoglobulin and rheumatoid factor, as well as secretion of factors which have further positive feedback effects on macrophages<sup>3</sup>. Similar findings of upregulated HLA expression, especially HLA-DR, have been made in other diseases, such as Graves disease and type 1 diabetes mellitus<sup>4</sup>. These findings led to the publication of a hypothesis suggesting that upregulation of HLA-DR expression and antigen presentation in the absence of adequate immunoregulation can trigger survival of autoreactive T cells that, , can lead to disease<sup>4</sup>. The search for cytokines that could upregulate HLA expression eventually led to the development of anti-TNF therapy for RA and other diseases<sup>5</sup>.

Immunohistological studies and studies using dissociated synovium showed that macrophages are the main TNF-producing cells in the inflamed RA joint, and their importance is illustrated by the efficacy of TNF inhibitors in disease therapy<sup>6</sup>. The mechanism underlying overproduction of TNF by synovial macrophages is poorly understood, but could involve cytokines, T lymphocytes, immune complexes (consisting of antigen bound by autoantibodies), Toll-like receptors or neutrophil extracellular traps<sup>7,8,9</sup>. Secretion of cytokines and chemokines perpetuates the inflammatory response by recruiting additional innate immune cells, such as monocytes and neutrophils, and also by inducing T-cell differentiation. IL-23, which is involved in RA<sup>10</sup>, induces population expansion and activation of IL-17-producing type 17 T helper cells (T<sub>H</sub>17) that are proposed to be important effectors in autoimmunity generally, and perhaps also in RA<sup>11</sup>. However, blockade of IL-17 signalling in RA is not as effective as anti-TNF therapy, suggesting the involvement of other effector pathways. Synovial fibroblasts are activated by cytokines derived from T cells and

macrophages, and contribute to feedback loops involved in the polarization and activation of macrophages<sup>7</sup>. In response to TNF and IL-1 $\beta$ , fibroblasts produce receptor activator of nuclear factor  $\kappa$ B (NF- $\kappa$ B) ligand (RANKL)<sup>12</sup> and macrophage colony-stimulating factor 1 (M-CSF, also known as CSF-1)<sup>13</sup>, which are essential for formation of osteoclasts, the resident macrophages of bone<sup>14,15</sup>. Macrophage-derived TNF, IL-6 and IL-1 can further amplify osteoclast functions, directly or indirectly<sup>16,17</sup>. In healthy individuals, bone resorption by osteoclasts is balanced by osteoblastic bone formation<sup>18</sup>, whereas in RA, osteoclast activity is chronically induced, causing severe bone erosion both focally, at cartilage–pannus junctions, and more diffusely<sup>19</sup>. Macrophages isolated from synovial tissue in RA constitutively secrete CXCL8 (also known as IL-8)<sup>20</sup> and monocyte chemoattractant protein 1 (CCL2, also known as MCP-1)<sup>21</sup>, which are important for neutrophil and monocyte recruitment.

Macrophages also contribute to disease progression by production of reactive oxygen species, nitric oxide intermediates and matrix-degrading enzymes, as well as by antigen presentation<sup>7</sup> (FIG. 1). Synovial fibroblasts also produce granulocyte-macrophage colony-stimulating factor (GM-CSF, also known as CSF2)<sup>22</sup>, which promotes arthritis, possibly by enhancing the survival and functioning of neutrophils and macrophages<sup>23</sup>. Macrophages are indeed one of the most abundant cell types at sites of inflammation in RA<sup>24</sup>, and clinically effective therapies have been shown to reduce macrophage numbers in affected joints<sup>25</sup>. In addition, total depletion of macrophages is beneficial for disease pathogenesis in animal models<sup>26</sup>. The evidence suggests, therefore, that macrophages have a central role in RA by orchestration of the cytokine environment, which enhances inflammation and contributes to the destruction of cartilage and bone.

#### **Heterogeneity of origin and function**

For many years, the prevailing dogma was that all macrophages are derived from circulating monocytes, so they are ultimately derived from progenitors in bone marrow. This view has been challenged by results published in the past 5 years from elegant lineage-tracing studies, indicating that embryonic precursors give rise to many tissue-resident macrophages<sup>27,28,29,30,31</sup>. Entirely for microglia in the brain, and to an extent, for Langerhans cells in the epidermis, tissue-resident macrophages are embryo-derived. In adulthood, these embryo-derived macrophage populations can maintain themselves by self-renewal<sup>32</sup>. By contrast, macrophages of the intestinal lamina propria and the dermis are entirely monocyte-derived, and are continually being replaced by monocytes from the circulation<sup>33,34</sup>. Most other tissues have a mixture of macrophages from both sources<sup>35</sup>. Notably, seeding of macrophages in the synovium and their capacity for self-renewal are yet to be formally addressed. The surface markers that are characteristic of murine tissue-resident macrophages are adhesion G protein-coupled receptor E1 (also known as cell-surface glycoprotein F4/80), tyrosine-protein kinase Mer, CD64 and CD14, whereas monocyte-derived cells infiltrating the tissues are more likely to express high levels of CD11b, CCR2 and lymphocyte antigen 6C2 (Ly-6C2), also known as Ly6C<sup>36,37</sup>.

### Embryonic haematopoiesis

During the development of mammalian embryos, haematopoiesis consists of a tightly regulated stepwise process involving several progenitor cell types, which begins in the yolk sac, passes through the fetal liver and ultimately ends up in the bone marrow at birth<sup>38</sup>. In developing murine embryos, primitive haematopoiesis occurs in the yolk sac around day 7 (E7.0) and generates macrophages and erythrocytes. This stage is later replaced by definitive haematopoiesis, occurring in the aorta–gonad–mesonephros region of embryonic mesoderm, which generates haematopoietic stem cells (HSCs) that populate the fetal liver at around E10.5<sup>39,40</sup>. Thus, the question remained whether embryonically derived tissue-resident macrophages have descended directly from primitive precursors in the yolk sac or from definitive HSCs in fetal liver. Conflicting data relating to the ontogenesis of tissue macrophages have resulted from the use of different fate-mapping approaches and mouse models. The controversy was resolved by the demonstration that tissue-resident macrophages are nearly all descendants of fetal HSCs or fetal monocytes (FIG. 2). A mouse strain was developed for conditional expression of a fluorescent marker in early yolk sac and fetal liver progenitors, fetal and adult HSCs, but not in mature haematopoietic cells<sup>41</sup>. This strain enabled the fate mapping of adult macrophages and myeloid cells at different times during ontogeny and demonstrated that all adult macrophages except for brain microglia and some epidermal Langerhans cells are derived from definitive haematopoiesis via HSCs<sup>41</sup>. A complementary approach combined *in utero* depletion of yolk-sac macrophages by antibodies to CSF-1 receptor (which is expressed in yolk-sac macrophages, but not in fetal monocytes) with a series of fate-mapping strains; the results led to a similar conclusion<sup>42</sup>. Fetal monocytes are derived from definitive erythromyeloid progenitors (EMPs), which express transcription factor c-Myb and which are phenotypically different from c-Myb negative primitive EMPs<sup>42</sup>. Other results have suggested that macrophage subsets of distinct origin can coexist in the same tissue; macrophages in the endocrine compartment of the pancreas (the islets of Langerhans) are derived from definitive haematopoiesis, whereas macrophage subsets in the exocrine compartment (the acinar glands) are derived from both primitive and definitive haematopoiesis<sup>43</sup> \_\_\_\_\_

### Postnatal haematopoiesis

Bone marrow becomes the main site of haematopoiesis after birth (FIG. 3). HSC progeny undergo stages of differentiation, losing developmental potential at every stage. Transcription factor p64 (proto-oncogene c-Myc) controls the balance between HSC self-renewal and differentiation; low levels of c-Myc ensure self-renewal and high levels induce differentiation toward a short-term HSC fate<sup>44</sup>. Continued differentiation leads to the loss of self-renewal activity, and early progenitor cells of different haematopoietic cell types become transient amplifying cells, which rapidly expand through proliferation while continuing to differentiate into increasingly lineage-restricted progenitors<sup>45,46</sup>. Firstly, common myeloid progenitors, which can generate all cells of the myeloid lineage, lose lymphoid potential. Next, granulocyte potential is lost when granulocyte myeloid progenitors give rise to monocyte–macrophage and dendritic cell

progenitors, which can develop into common monocyte progenitors or into common dendritic cell progenitors. The existence of monocyte–macrophage and dendritic cell progenitors is not universally accepted, and the results of a study that combined clonal culture assays with adoptive transfer experiments found no evidence for their existence<sup>47</sup>, but instead suggested the presence of oligopotent myeloid progenitors<sup>47</sup>. Although the branch point between common monocyte progenitors and common dendritic cell progenitors is debatable, common monocyte progenitors clearly give rise to monocyte-derived macrophages<sup>48</sup>, as common dendritic cell progenitors give rise to resident dendritic cells<sup>49, 50</sup>. The spleen contains a large pool of monocytes that can be recruited during acute inflammation<sup>51, 52</sup>. Two major monocyte subsets have been identified in mice: ‘classical’ (Ly6C<sup>hi</sup> CX3CR1<sup>int</sup> CCR2<sup>+</sup>); and ‘nonclassical’ (Ly6C<sup>lo</sup> CX3CR1<sup>hi</sup> CCR2<sup>-</sup>), where “hi”, “int” and “lo” refer to high, intermediate and low levels of expression, respectively<sup>53</sup>. Ly6C<sup>hi</sup> monocytes, which are also referred to as inflammatory monocytes, require CCR2 expression for egress from the bone marrow<sup>54, 55</sup>. The inflammatory signature of Ly6C<sup>hi</sup> monocytes and their progeny during inflammation is regulated by GM-CSF, a proinflammatory cytokine involved in terminal differentiation of myeloid cells and implicated in many autoimmune diseases, including RA<sup>23</sup>. Ly6C<sup>hi</sup> monocytes are required for the initiation of tissue inflammation<sup>56</sup>. Ly6C<sup>lo</sup> monocytes, which are less prevalent in the circulation than the Ly6C<sup>hi</sup> subset, patrol the vasculature and survey endothelial integrity<sup>57</sup>. Evidence suggests that Ly6C<sup>hi</sup> monocytes give rise to Ly6C<sup>lo</sup> monocytes, both in bone marrow and in the circulation<sup>30, 48</sup>. In humans, three subsets of monocytes have been defined: classical (CD14<sup>++</sup> CD16<sup>-</sup>); intermediate (CD14<sup>++</sup> CD16<sup>+</sup>); and nonclassical (CD14<sup>+</sup> CD16<sup>++</sup>)<sup>58</sup>. The classical and nonclassical human monocytes are homologous to their classical and nonclassical murine counterparts. However, although murine and human monocyte subsets are similar, they display species-specific differences in gene-expression profiles<sup>59</sup>.

Available information relating to the embryonic versus adult bone-marrow origin of tissue macrophages, and to the contribution of local proliferation to sustaining macrophage numbers, refers exclusively to the mouse, and little is known of the origin of human macrophages. Moreover, although proliferation-associated genes are expressed during human monocyte-to-macrophage differentiation<sup>60</sup>, proliferative activity of human macrophages has not yet been fully assessed.

### **Monocytes and macrophages**

The discovery that tissue-resident macrophages are seeded prior to birth raises questions about the biology of monocytes both in the steady state and in inflammation. Both murine monocyte subsets have been reported to enter tissues in the steady state for immune surveillance, although the presence of Ly6C<sup>lo</sup> monocytes in normal tissues has been questioned<sup>53, 61, 62</sup>. Upon tissue entry, monocytes can differentiate to macrophages by upregulating expression of macrophage-associated genes<sup>32</sup>. This process is especially prominent in response to macrophage depletion, inflammatory conditions or physiological stress. In the steady state, some embryonically derived resident cells, such as cardiac macrophages, are replaced by macrophages derived from Ly6C<sup>hi</sup> monocytes during postnatal development<sup>29, 63</sup>. However, monocytes can

also remain undifferentiated in the tissue, survey the environment, elevate the expression of MHCII due to their interaction with endothelium and cytokines and acquire antigen for carriage to draining lymph nodes<sup>61</sup>. Thus, the accumulated evidence suggests that differentiation to macrophages is not obligatory after entry of monocytes into tissue and triggers are necessary to initiate differentiation. The intestine, where macrophages are constantly being derived from monocytes in the steady state, is continuously exposed to such triggers in the form of the microbiota and its products.

The newly appreciated heterogeneity of the origin of tissue-resident macrophages poses an intriguing question as to whether monocyte-derived macrophages can fully replicate the phenotype and function of embryonically derived macrophages. In fact, a new study has directly addressed this question by transferring adult monocytes into the empty alveolar macrophage niche of neonatal *Csf2rb* deficient animals and demonstrating that they can colonise this empty niche and develop into functional tissue-resident macrophages<sup>64</sup>. Similarly, transferred yolk sac of fetal liver monocytes can efficiently colonise the alveolar macrophage niche, indicating that precursor origin does not affect the development of functional tissue-resident macrophages, but mature macrophages derived from different tissues fail to do so. Gene-expression profiling of monocyte-derived alveolar macrophages demonstrated that with a noticeable exception of the genes coding for proteins in the MHCII complex, they were indistinguishable from true tissue resident alveolar macrophages. Improvements in our understanding of the molecular mechanisms controlling macrophage adaptation to tissue-specific environments, and molecular regulation of macrophage phenotype and function, are required to elucidate the full functions of macrophages and to facilitate interventions to regulate them.

#### **Regulatory factors and epigenetics**

Innovative genome-wide studies of macrophage transcriptional regulation have demonstrated a hierarchical organisation of the transcriptional networks, involving a combination of transcription factors specific to macrophage lineage and to the tissue microenvironment. The function of tissue-resident macrophages in disease or other challenge is further controlled by stimulus-specific transcription factors that respond to environmental signals.

#### **Transcriptional regulation**

Transcription factor PU.1 (encoded by the *Spi1* gene in mice) has been implicated as a top-level regulator throughout haematopoietic development of myeloid lineages<sup>65, 66</sup> (FIG. 4). CSF-1 drives steady-state proliferation of resident tissue macrophages and can directly stimulate activation of the *Spi1* promoter increasing the number of PU.1-expressing cells with a myeloid gene signature and differentiation potential<sup>67</sup>. PU.1 activates expression of Kruppel-like factor 4, which can induce exclusively monocytic differentiation of common myeloid progenitors via upregulation of CD14<sup>68, 69</sup>. PU.1 also regulates expression of early growth response protein 1 (EGR-1) and EGR-2, which counteract the neutrophil fate of precursors by activating macrophage-specific genes and repressing the neutrophil programme<sup>70</sup> (FIG. 4a).

PU.1 not only directs development of monocytes throughout haematopoiesis, but also regulates the genomic landscape of macrophages, binding to macrophage-specific enhancer elements and preventing their masking by nucleosomes<sup>71, 72, 73</sup>. PU.1 interacts with other macrophage-specific transcription factors, such as CCAAT/enhancer-binding protein  $\alpha$  (C/EBP $\alpha$ ), which contribute to the specificity of genomic recruitment<sup>73, 74</sup>. Within the myeloid compartment, interferon regulatory factor 8 (IRF-8) is expressed from the granulocyte myeloid progenitor stage onward, and is selective for the monocyte–macrophage branch of differentiation<sup>75</sup>. In resting cells, IRF-8 mainly acts with PU.1 to maintain the expression of a number of genes essential for macrophage functions, such as microbial recognition and response to purines<sup>76</sup>.

### **Environmental regulation**

Factors derived from the tissue environment influence the phenotype of tissue macrophages via induction of tissue-specific transcription factors. For example, in the peritoneum, retinoic acid promotes the expression of transcription factor GATA-6, which controls the peritoneal macrophage-specific phenotype and proliferative renewal<sup>77, 78</sup>. Likewise, GM-CSF has a lung-specific role in the perinatal development of alveolar macrophages through the induction of peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ) in fetal monocytes<sup>79</sup>. Environmental signals regulate tissue-specific transcription factors that interact with PU.1 at tissue-specific enhancers<sup>80, 81</sup>. Nearly all tissue-resident macrophages have unique patterns of enhancer utilization. For example, intestinal macrophages, which are mostly monocyte-derived, have more enhancers in common with monocytes than with other tissue-resident macrophages<sup>81</sup>. Macrophages that are embryonically derived are characterized by different enhancers, and microglia and lung macrophages are most distant from other tissue-resident subsets. However, after transplantation of adult bone-marrow precursors into adult animals, tissue-specific enhancer utilization patterns can be recapitulated in monocyte-derived macrophages, highlighting a prominent role of the microenvironment in establishing macrophage identity<sup>81</sup>. In addition, when differentiated tissue-resident peritoneal macrophages are transferred into the lung microenvironment, their gene expression profile switches to resemble that of lung macrophages, including downregulation of GATA-6, emphasizing the plasticity that is retained by the differentiated macrophages<sup>81</sup>. Further evidence for environment-specific macrophage specialization is provided by the diversity of macrophage subsets observed in different anatomical locations in the pancreas<sup>43</sup>. Macrophages in islets of Langerhans and in the interacinar stroma are distinct in both origin and phenotypic characteristics, and complete replacement of islet and stromal macrophages by donor stem cells results in the donated cells adopting identical profiles to the original macrophages<sup>43</sup>.

### **Macrophage polarization**

PU.1-associated macrophage-specific enhancers can be active or latent in the resting cell state<sup>82</sup>, but they are targeted by signal-specific transcription factors in response to environmental challenges, inflammation or physiological stress. In macrophages induced by lipopolysaccharide treatment, NF- $\kappa$ B, transcription

factor AP-1 and members of the IRF and signal transducer and activator of transcription (STAT) families bind to PU.1-marked enhancers and contribute to regulation of gene expression<sup>72, 73, 83</sup>. IRF-5 is implicated in the establishment of an inflammatory macrophage phenotype in the presence of GM-CSF or interferon  $\gamma$ <sup>84, 85</sup>, and specifically binds to PU.1-marked enhancers and promoters of inflammatory genes. IRF-5 requires NF- $\kappa$ B, and possibly PU.1 itself, for efficient docking and function<sup>86</sup> (FIG. 4a). In response to diverse environmental stimuli, such as interferon  $\gamma$  (or lipopolysaccharide) and IL-4 (or IL-13), macrophages have been proposed to undergo activation or polarization programmes, which have been classified by several different systems of nomenclature<sup>87</sup>. Historically, several functional properties distinguish M1 (classically activated) and M2 (alternatively activated) polarized macrophages, including the repertoires of cytokines (IL-12<sup>hi</sup> IL-23<sup>hi</sup> IL-10<sup>lo</sup> versus IL-12<sup>lo</sup> IL-23<sup>lo</sup> IL-10<sup>hi</sup>), chemokines (CXCL9 and CXCL10 versus CCL17 and CCL22), micro-RNA (miR-155 versus miR-223), iron, glucose and folate metabolism, scavenger receptors and mannose receptors<sup>87, 88, 89, 90</sup> (TABLE 1). M1 polarized macrophages are generally involved in resistance to intracellular pathogens and to tumours in the context of T<sub>H</sub>1-driven responses, whereas M2 polarized macrophages are effectors of resistance to parasites, have immunoregulatory properties, promote tumour growth and invasiveness and orchestrate tissue repair and remodelling, including fibrosis<sup>91, 92</sup>. Transcription factors involved in macrophage polarization include NF- $\kappa$ B, Stat1 and IRF-5 in the M1 pathway, and IRF-4, Stat6, c-Myc, PPAR- $\gamma$  and krueppel-like factor 4 in the M2 pathway<sup>93</sup> (FIG. 4; TABLE 1). The M1 and M2 states represent extremes on a continuum, and many intermediate and diverse phenotypes have also been described<sup>87, 94</sup>.

The observed microenvironmental dominance and the ability of macrophages derived from monocytes to adopt the phenotype of tissue-resident macrophages enable the recruitment of monocytes in situations such as infection, ageing or pathologies, where self-renewal of tissue-resident macrophages might not be sufficient to sustain macrophage replenishment or function<sup>95</sup>. Notably, depletion of activated monocytes by repeated leukapheresis in patients with active RA leads to improvements in rheumatoid activity that persists for 10-12 weeks<sup>96</sup>. It also leads to repopulation with monocytes that have a reduced activation status with respect to cytokine and inflammatory mediator production<sup>97</sup>.

### **Macrophages in the synovium**

The contribution of monocytes to the macrophage populations in the joints, as well as the origin of resident synovial macrophages, has not been extensively characterized. Immunohistological examination of frozen sections of pannus tissue taken from the joints of patients with RA has revealed that the phenotypes of macrophages in the synovial lining differ from the phenotype of macrophages accumulating at the cartilage junction<sup>98</sup>. In RA joints, accumulation of monocytes and macrophages positive for protein S100-A8 (MRP-8) and S100-A9 (MRP-14) was observed in the sublining layer, whereas mature macrophages positive for late inflammatory macrophage markers were more abundant in the lining layers<sup>99</sup>, indicating a distinction between infiltrating (sublining) and tissue-resident (lining) macrophage phenotypes. The lining

macrophages in patients with spondyloarthritis expressed higher levels of the IL-10 and M2 polarization marker CD163 relative to patients with RA, but no differences were found in other M1 and M2 markers between the diseases<sup>100</sup>. Although lining macrophages had an IL-10 phenotype, synovial sublining macrophages had a more heterogeneous phenotype, with a majority of cells coexpressing M1 and M2 markers<sup>100</sup>. Evidence is lacking for macrophage polarization in either direction in the inflamed joint, but TNF and IL-1, which are characteristically released in higher quantities by M1 macrophages, are known to be abundant in RA, whereas IL-10 activity, which is characteristic of M2 macrophages, is relatively diminished, compared with unaffected individuals<sup>101</sup>. Mice deficient in IL-10 develop exacerbated inflammatory arthritis, and the phenotype of synovial macrophages in these animals is shifted towards the proinflammatory M1 state<sup>102</sup>. Treatment of patients with anti-TNF therapy has a rapid and pronounced effect on the infiltration of MRP-positive macrophages into tissues<sup>103</sup>, suggesting an important role for infiltrating macrophages as opposed to resident tissue macrophages in human autoimmune synovitis.

### **Ly6C<sup>lo</sup> monocyte infiltration**

The model of sterile inflammatory arthritis induced by serum from K/BxN mice recapitulates the effector phase of human RA, but is independent of the adaptive immune response<sup>104</sup>. The model relies on immune-complex engagement of activation Fc receptors on macrophages, and intravenous  $\gamma$  globulin (IVIG) protects mice from the disease through expression of inhibitory Fc receptors<sup>105</sup>. However, mice deficient in CSF-1 are not protected by IVIG, indicating the presence of both CSF-1-dependent and CSF-1-independent macrophage subsets<sup>105</sup>. Results of experiments with the K/BxN model have demonstrated that circulating Ly6C<sup>lo</sup> monocytes are recruited to the ankle joint during the effector stage of the induced disease, and that they differentiate into classically activated macrophages to drive the pathology<sup>106</sup> (FIG. 5a). In these experiments, monocytes in the peripheral blood and spleen were depleted by clodronate-loaded liposome treatment, which completely prevented the development of arthritis and suppressed the recruitment of neutrophils to the joint. Results with anti-CCR2 antibodies and *Ccr2*<sup>-/-</sup> mice demonstrated that reduction or elimination of Ly6C<sup>+</sup> monocytes did not influence disease progression<sup>106</sup>. In unchallenged ankle joints in wild-type mice, the resident macrophages in the synovial lining comprised two different populations, MHCII<sup>-</sup> CX3CR1<sup>hi</sup> (80%) and MHCII<sup>+</sup> CX3CR1<sup>lo</sup> (20%)<sup>106</sup>. The MHCII<sup>-</sup> macrophages were radioresistant, long lived, and CSF-1 dependent, indicating that they are derived from embryonic progenitors. Thus, tissue-resident MHCII<sup>-</sup> macrophages seem to populate the synovial tissue early during embryonic development and have an important role in maintaining joint integrity; specific intra-articular depletion of these cells led to the development of accelerated arthritis in the K/BxN model<sup>106</sup>. By contrast, the MHCII<sup>+</sup> macrophages originated from bone marrow and required a contribution from circulating monocytes<sup>106</sup>. Intra-articular depletion of MHCII<sup>+</sup> tissue-resident macrophages without affecting the circulating monocyte pool had no effect on the course of the K/BxN induced disease, whereas a systemic depletion of both circulating monocytes and bone-marrow-derived synovial macrophages markedly reduced the severity of the induced disease<sup>106</sup>. In this system, the depletion of circulating monocytes at the peak of arthritis did not affect the

resolution phase of the disease, but the depletion of macrophages that had already infiltrated the joint delayed the resolution of arthritis<sup>106</sup>. These results, together with profiling of macrophage gene-expression repertoires, suggest that recruited macrophages undergo a reprogramming of their inflammatory phenotypes at the later stages of the disease, contributing to the resolution of arthritis.

### Ly6C<sup>hi</sup> monocyte infiltration

The characterization of synovial macrophages was further extended by the comparison of macrophage populations in naive ankle and knee joints<sup>107</sup>. Although the results were generally similar in the two locations, some differences between macrophage populations were also noted, such as a higher representation of MHCII<sup>+</sup> macrophages in the knee than in the ankle. However, contrary to results with the KBxN model of arthritis, in an antigen-induced arthritis (AIA) model, which mimics both the induction and effector phases of arthritis<sup>104</sup>, >90% of infiltrating monocytes were Ly6C positive and the majority were Ly6C<sup>hi</sup><sup>107</sup> (FIG. 5b). In this model, monocyte recruitment was significantly reduced in the affected joints of *Ccr2*<sup>-/-</sup> mice relative to wild-type, indicating that Ly6C<sup>hi</sup> monocytes were the predominant source of infiltrating monocytes. Furthermore, Ly6C<sup>hi</sup> monocytes were the most likely source of inflammatory macrophages in the knee, which were absent in *Ccr2*<sup>-/-</sup> mice<sup>107</sup>. Thus, despite somewhat similar joint pathologies, the two models clearly differ in the types of circulating monocytes recruited to the joint. This difference is likely to be because of the immunization in AIA with complete Freund's adjuvant, which is composed of inactivated and dried mycobacteria that could predispose mice to preferentially recruit classical Ly6C<sup>hi</sup> monocytes to the site of inflammation.

Another model of joint inflammation that relies on immunization with complete Freund's adjuvant is collagen-induced arthritis (CIA), which is the gold standard for murine studies because of the similarities with human RA, including breach of self-tolerance and generation of autoantibodies, as well as excessive production of inflammatory cytokines<sup>104</sup>. Results from a number of studies have directly or indirectly proved that Ly6C<sup>hi</sup> CCR2<sup>+</sup> monocytes cause autoimmune arthritis during CIA. A genetically engineered suppressor protein that blunted CCR2-dependent cellular signalling was delivered into mice at the onset of arthritis and caused a robust clinical recovery and a significant reduction in anti-collagen antibody titre and rheumatoid factor titre, as well as reduction of both intra-articular and systemic proinflammatory cytokine levels<sup>108</sup>. Silencing of nicotinamide phosphoribosyltransferase (*Nampt*) expression in Ly6C<sup>hi</sup> monocytes at the onset of arthritis, using intravenous injection of small interfering RNA (siRNA) against *Nampt*, inhibited CIA progression. This effect was associated with reductions in IL-6 production by Ly6C<sup>hi</sup> monocytes, the proportion of T<sub>H</sub>17 cells and autoantibody titres, as well as the activation and infiltration of monocytes, macrophages and neutrophils in arthritic joints<sup>109</sup>. Depletion of Ly6C<sup>+</sup> monocytes and macrophages, triggered by antibodies against death receptor 5, resulted in prevention of the development of CIA, or amelioration of the severity of CIA, when administered before or after the onset of arthritis, respectively<sup>110</sup>. Depletion of Ly6C<sup>hi</sup> monocytes with the monoclonal antibodies against *Ccr2*

ameliorated CIA<sup>111</sup>, in contrast mice deficient in Ccr2 receptor displayed accelerated arthritis in CIA model, suggesting a possible effect on development or function of other immune cell types<sup>112</sup>. Notably, the intermediate CD14<sup>++</sup> CD16<sup>+</sup> human blood monocytes (corresponding to Ly6C<sup>int</sup> in mouse) are observed at higher frequency in patients with RA than in healthy individuals, whereas nonclassical CD14<sup>+</sup> CD16<sup>++</sup> monocytes (Ly6C<sup>lo</sup> in mouse) are unchanged in RA<sup>113</sup>. Despite the accumulation of data concerning macrophage origin in mouse models of arthritis, the marked difference in the results in different forms of arthritis indicates that extrapolation to human RA or other forms of arthritis is premature.

### **The development of antirheumatic drugs**

Current baseline treatment for RA involves DMARDs, most commonly methotrexate, and steroids or NSAIDs<sup>114</sup>. These drugs are not specific for macrophages, but do mitigate some of the effects of macrophage activation, such as the production of important macrophage mediators, including TNF and IL-6. However, this response is only achieved with doses of corticosteroids that are potentially toxic if sustained for more than a few days. Macrophages produce large amounts of proinflammatory cytokines. The first and most widely used biologic antirheumatoid drugs, the anti-TNFs, target TNF, which in RA is mainly produced by macrophages. The extensively used TNF blockade employs either monoclonal antibodies (infliximab, adalimumab, certolizumab or golimumab) or a TNF receptor 2 (p75)-human IgG1 fusion protein (etanercept). Blockade of other cytokines can also be beneficial, including IL-6; tocilizumab is a clinically effective antibody directed against the IL-6 receptor. Antibodies to IL-6 have been effective in phase 2 clinical trials<sup>115</sup>. Blockade of IL-1 is also effective, but less so than other treatments, and is not in routine clinical use.

### **Targetting inflammatory macrophages**

No therapy has yet been shown to be efficacious and safe for specific elimination of inflammatory macrophages in RA. Selective elimination of synovial inflammatory macrophages in RA with a CD64-directed immunotoxin has been proposed<sup>116</sup>. In patients with RA, compared with monocytes from peripheral blood, inflammatory macrophages from synovial fluid express elevated levels of CD64, and a CD64-directed immunotoxin could promote their selective elimination via apoptotic cell death<sup>116</sup>. In cultures of mononuclear cells obtained from patients with RA, induction of synovial fluid macrophage apoptosis was associated with efficient inhibition of antigen-induced lymphocyte proliferation, and a reduction in secretion of TNF and IL-1 $\beta$ . Moreover, depletion of CD64<sup>+</sup> macrophages had therapeutic effects in an *in vivo* adjuvant arthritis model using human *FCGR1A* transgenic rats<sup>117</sup>. After induction of adjuvant arthritis, treatment with CD64-directed immunotoxin resulted in a significant reduction in macrophage numbers, followed by diminished inflammation and bone erosion in paws<sup>117</sup>. The chemotherapeutic agent trabectedin, approved by the FDA in 2015 for treatment of liposarcoma and

leiomyosarcoma<sup>118</sup> <http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm468832.htm> (2015).], induces rapid apoptosis exclusively in mononuclear phagocytes, causing selective depletion of monocytes and macrophages in blood and tissues<sup>119</sup>. This drug may be worth testing in RA although toxicity might limit its usefulness given the precedent of antitumor drugs (eg MTX) turning out to be useful in RA,

#### **Targeting circulating monocytes**

Current evidence strongly suggests that circulating monocytes that infiltrate the joint during inflammation, as well as their progeny, can be appropriate targets for therapeutic intervention in arthritis. Infused, negatively charged, immune-modifying microparticles (derived from polystyrene, microdiamonds or biodegradable poly(lactic-co-glycolic acid)) are efficiently taken up by inflammatory monocytes<sup>120</sup>. Subsequently, these monocytes are no longer trafficked to sites of inflammation, and disease symptoms show reductions in mouse models including myocardial infarction, experimental autoimmune encephalomyelitis, colitis induced by dextran sodium sulfate and thioglycollate-induced peritonitis<sup>120</sup>. Examination of the effectiveness of immune-modifying microparticles in models of inflammatory arthritis could prove very interesting. However it is worth noting that human clinical trials using anti-CCR2 blocking antibody did not result in amelioration of synovial inflammation in active RA<sup>121</sup>, possibly due to the redundancy receptors involved in monocyte recruitment to the site of inflammation in humans<sup>122</sup>.

#### **Targeting GM-CSF**

Considerable interest exists in novel targets that could be manipulated to change the proinflammatory monocyte and macrophage phenotype, such as CCR2, DR-5 and NAMPT. GM-CSF signalling in CCR2<sup>+</sup> myeloid cells is necessary for the pathogenesis of experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis<sup>56</sup>, and is likely to have a role in other inflammatory diseases, including RA. In fact, in CIA, a dose-dependent reduction of clinical arthritis scores and the number of F4/80-positive macrophages in the inflamed synovium was observed after treatment with an anti-GM-CSF-receptor (GM-CSF-R) antibody<sup>123</sup>. Therapies targeting GM-CSF, both by GM-CSF depletion and GM-CSF-R blockade, have been developed and are being evaluated in preclinical studies and clinical trials<sup>124</sup>. Preclinical studies demonstrated that the number of GM-CSF-R-positive cells is significantly higher in synovium from patients with RA than from those with osteoarthritis or healthy controls<sup>123</sup>. The GM-CSF-R antagonist mavrilimumab has shown promising results in patients with RA in phase 2 trials<sup>125</sup>. By contrast, CSF-1 blockade has not yet been successful in clinical trials for RA<sup>126</sup>, despite showing beneficial therapeutic effects in mouse models of arthritis<sup>23, 127</sup>.

#### **Targeting IRF-5**

GM-CSF induces expression of many genes, including those encoding chemokines, co-stimulatory molecules and regulatory factors<sup>56</sup>. Regulation of expression of transcription factor IRF-5 occurs downstream of GM-

CSF-R signalling in both human and mouse monocytes<sup>84, 85</sup>. In both ankle and knee joints, the populations of MHCII<sup>+</sup> macrophages are severely compromised in IRF-5-deficient naive animals<sup>107</sup>. IRF-5 deficiency limits neutrophil influx into the inflamed knee in the early stages of AIA, reducing swelling of the knee and leading to a reduction in the pathogenic T<sub>H</sub>1, T<sub>H</sub>17 and  $\gamma\delta$  T (IL-17<sup>+</sup>) cells in the joint at the later stages of the disease<sup>107</sup> (FIG. 6). The presence of IRF-5 seems to be important for differentiation of monocytes into CD64<sup>+</sup> macrophages, and for establishment of the MHCII<sup>+</sup> inflammatory macrophage phenotype in the arthritic joint, and the secretion of neutrophil attractants, such as chemokine CXCL1<sup>107</sup>. Notably, *Irf5*<sup>-/-</sup> mice undergoing the K/BxN serum-transfer model of arthritis also display reduced disease severity and lower levels of chemokines in the serum relative to wild-type mice<sup>128</sup>. Furthermore, the influx of IRF-5<sup>+</sup> monocytes and macrophages into the affected knee is completely abolished in *Ccr2*<sup>-/-</sup> animals<sup>107</sup>. The evidence suggests that IRF-5 is a transcription factor that is involved in GM-CSF signalling to establish the inflammatory signature of Ly6C<sup>hi</sup> monocytes<sup>129</sup>, so IRF-5 blockade might be an effective therapeutic target in RA. Blocking IRF-5 might not be easy, as transcription factors are often considered not to be 'druggable', but nanoparticle delivery of *Irf5* siRNA has been achieved in murine cardiac macrophages, reducing expression of inflammatory M1 macrophage markers, supporting resolution of inflammation, accelerating cutaneous and infarct healing and attenuating development of postmyocardial-infarction heart failure after coronary ligation, relative to delivery of control siRNA<sup>130</sup>. Macrophage phenotype has also been manipulated by nanoparticle-mediated delivery of a plasmid encoding IL-10 to macrophages of arthritic rats, resulting in a repolarization of macrophage phenotype and prevention of the progression of inflammation and joint damage<sup>131</sup>.

Many opportunities exist for the development of new therapies by targeting macrophages. Whether any of these therapies is more effective than the current standard of care for severe RA — namely, the combination of biologics and DMARDs — and at the same time is at least as safe remains to be seen.

### Future perspectives

Advances in single-cell methodologies, such as single-cell RNA sequencing and mass cytometry, enable unbiased identification of the molecular state of individual cells<sup>132, 133, 134</sup>. Even for a seemingly homogeneous cell population, such as bone-marrow-derived dendritic cells, substantial variation between identically stimulated cells has been detected, in both the fraction of cells detectably expressing a given mRNA and the transcript level within expressing cells<sup>132</sup>. Notably, preventing cell-to-cell communication substantially reduces variability between cells in the expression of an early-induced inflammatory module. For tissue-resident immune cells, distinct states and cell types can be classified by single-cell analysis, enabling a detailed appreciation of cellular heterogeneity<sup>133</sup>. Proteomic measurements are also important in macrophage immune profiling, as the global correlation between population measurements of mRNA and protein levels is weak<sup>135</sup>. Moreover, single-cell proteomic measurements are essential for optimal understanding of cellular signalling encoded in posttranslational protein modifications. The use of mass

cytometry to profile the mouse myeloid system has confirmed a clear separation of phenotypes of tissue-resident macrophages among tissues in the steady state<sup>136</sup>. Thus, the application of 'omics' technologies at the single-cell level can provide powerful tools to uncover dysregulated pathways and the immune-cell subsets that are involved in disease in individual patients. Single-cell omics applications should be ideally suited to the analysis of clinical samples, as they only require minute amounts of biological material<sup>137</sup>.

### Conclusions

Macrophages are important immune cells that are involved in both initiation and resolution of inflammation. They are of critical importance in rheumatoid arthritis (RA), where they generate cytokines that enhance inflammation and contribute to destruction of cartilage and bone. While multiple studies support the notion that both embryonically derived macrophages and macrophages derived from adult bone marrow are present in most tissues, the origin of macrophages in human tissue, including the synovium, is not yet known. The microenvironment plays the key role in determining identity and function of tissue-resident macrophages and importantly bone-marrow-derived macrophages are capable of adopting the phenotypes of tissue-resident macrophages and develop into functional tissue-resident macrophages. Tissue-resident macrophages adopt distinct transcriptional profiles in response to specific microenvironments<sup>80, 81</sup>, and widespread changes occur in macrophage gene expression in response to inflammatory challenges<sup>94</sup>. However, at the individual cell level, the heterogeneity of the macrophage response *in vivo* during inflammation has not yet been addressed. Single-cell methodologies not only provide the basis for the delineation of developmental and activation programmes of immune cells, but also hold the promise of identifying specific macrophage subsets for directed therapeutic intervention, without interfering with host protection or resolution pathways. Targeting specific macrophage subsets can present an attractive new therapeutic strategy for RA, but this needs to be reintegrated into a wider understanding of the aspects of disease and the role of such factors as age, sex, genetics, diet, microbiome and drug treatment, that differ between the patients. Only then will the prospect of therapies with improved efficacy and safety be fully realized, and we might get close to a cure.

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### Author contributions

I.A.U. researched the data for the article. I.A.U. and M.F. contributed substantially to discussions of the article content and wrote the manuscript. All authors (I.A.U., A.M. and M.F.) undertook review or editing of the manuscript before submission.

### Competing interests

The authors declared no competing interests.

### Key points

- Macrophages are of critical importance in rheumatoid arthritis (RA), where they generate cytokines that enhance inflammation and contribute to destruction of cartilage and bone
- Most murine tissues include both embryonically derived macrophages that can self-renew and macrophages derived from adult bone marrow, but the origin of macrophages in human tissues is not yet known
- The environment is very important in determining the unique identity and function of tissue-resident macrophages through the regulation of transcription factors and establishment of tissue-specific enhancers
- Bone-marrow-derived macrophages are capable of adopting the phenotypes of tissue-resident macrophages
- Seeding and self-renewal of macrophages in the mouse synovium are yet to be determined, but immunophenotyping indicates that macrophages in the synovial lining comprise embryonically derived and bone-marrow-derived populations
- Future therapies for RA could target specific macrophage subsets, although whether such therapies would interfere with host protective or resolution pathways is not yet known

Figure 1 | **The role of macrophages in RA.** Macrophages produce cytokines, which in turn promote inflammation by recruitment of additional immune cells, T-cell polarization and fibroblast activation. Activated fibroblasts secrete receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) and macrophage colony-stimulating factor 1 (M-CSF), inducing osteoclast differentiation, which is enhanced by macrophage-derived TNF and other cytokines. Immune complexes formed by autoantibodies and antigens activate macrophages. Additionally, macrophages are influenced by cell–cell contact or by cytokines produced by T-cells, fibroblasts and innate immune cells. MCP-1, monocyte chemoattractant protein 1;  $T_H1$ , type 1 T helper cells;  $T_H17$ , type 17 T helper cells.

Figure 2 | **Embryonic origins of tissue-resident macrophages.** The majority of tissue-resident macrophages are seeded during embryonic development. Yolk-sac macrophages arise first from primitive haematopoiesis

and seed embryonic tissues including the brain as early as E9.0. A wave of yolk-sac-derived erythromyeloid progenitors (EMPs) seeds the fetal liver, where definitive haematopoiesis is established on E11.0. The fetal liver is the main site of haematopoiesis until birth. On E12.5, fetal-liver-derived monocytes populate the embryo and dilute the yolk-sac-derived populations. However, yolk-sac macrophages remain the sole source of microglia, the brain's resident macrophages, likely because of the prior establishment of the blood–brain barrier. In epidermis and pancreas, macrophages seem to have a hybrid origin, arriving from both yolk sac and fetal liver waves. In other tissues, resident macrophages are exclusively derived from fetal-liver monocytes. The fate mapping of tissue-resident synovial macrophages has not yet been done.

**Figure 3 | Myelopoiesis in the adult bone marrow and spleen.** After birth, the bone marrow becomes the predominant site of definitive haematopoiesis, which relies on self-renewing haematopoietic stem cells (HSCs) constantly generating progenitors. The myeloid lineage derives from the common myeloid progenitor (CMP), which gives rise to the granulocyte–macrophage progenitor (GMP). Granulocytes arise from additional precursors not shown in this overview. Neutrophils are the most abundant granulocytes residing in the bone marrow until release into the blood stream. The monocyte and dendritic cell progenitor (MDP) gives rise to the common dendritic cell progenitor (CDP) and common monocyte progenitor (cMoP), and possibly to some granulocytes. CDPs develop into plasmacytoid dendritic cells (pDCs) or pre-DCs, the precursors of the conventional dendritic cells (cDC1 and cDC2). Two monocyte subsets originate from the cMoP, although recent data suggests that the Ly6C<sup>hi</sup> subset progresses into Ly6C<sup>lo</sup> monocytes. The spleen is a site of extramedullary monocytopoiesis where MDPs, cMoPs and both monocyte subsets have been detected. Monocytes circulate in the blood and upon tissue entry can differentiate into macrophages and DCs.

**Figure 4 | PU.1 in the transcriptional control of macrophage development and activation.** a | Runt-related transcription factor 1 (RUNX1) is required during early development in haematopoietic stem cells (HSCs), and is followed by the onset of PU.1 expression. PU.1 is the master regulator of the myeloid lineage and is crucial throughout differentiation. Interferon regulatory factor 8 (IRF-8), early growth response protein 1 (EGR-1) and EGR-2 are involved in suppressing granulocyte and neutrophil genes while promoting macrophage fate. Krueppel-like factor 4 (KLF4) is required for monocyte/macrophage differentiation. During inflammation, macrophages are shaped by the cytokine environment, which depends on the nature of the infectious insult. Bacterial pathogens cause a type 1 T helper cell (T<sub>H</sub>1) or T<sub>H</sub>17 response, which leads to signal transducer and activator of transcription 1 (STAT1) and interferon regulatory factor 5 (IRF-5) activation (classically activated M1 phenotype). Parasites lead to a T<sub>H</sub>2 response activating STAT6 and IRF-4 in macrophages (alternatively activated M2 phenotype). *In vivo*, multiple macrophage phenotypes exist along a continuum with M1 and M2 at the extremes. b | Typical composition of PU.1-marked enhancers and promoters in classically activated M1 macrophages. Binding of STAT-1 is associated with latent but not constitutive or poised enhancers<sup>82</sup>. The chromatin modification histone H3 lysine 4 mono-methylation

(H3K4me1) is associated with active enhancer elements, whereas histone H3 lysine 4 tri-methylation (H3K4me3) is associated with active promoters. DC, dendritic cell; NF-κB, nuclear factor κB.

**Figure 5 | The role of Ly6C<sup>hi</sup> and Ly6C<sup>lo</sup> monocytes in the development of different murine arthritis models.** a | Ly6C<sup>lo</sup> monocytes drive the development of sterile inflammatory arthritis. Transfer of K/BxN serum causes a CCR2-independent influx of Ly6C<sup>lo</sup> monocytes, which give rise to MHCII<sup>+</sup> inflammatory macrophages. These polarize towards an alternatively activated phenotype (MHCII<sup>-</sup>) during the development of arthritis. Tissue-resident synovial macrophages (largely MHCII<sup>-</sup>) limit the development of arthritis. b | Ly6C<sup>hi</sup> monocytes drive the development of antigen-induced inflammatory arthritis. Methylated bovine serum albumin (mBSA) challenge causes a CCR2-dependent influx of Ly6C<sup>hi</sup> monocytes, which in turn leads to an increase in inflammatory MHCII<sup>+</sup> macrophage numbers. Proportions of anti-inflammatory CD206<sup>+</sup> macrophages are decreased in inflamed knees compared with controls treated with phosphate-buffered saline (PBS).

**Figure 6 | Lack of IRF-5 causes a reduction in neutrophil influx and macrophage-derived CXCL1 in the arthritic knee.** In an antigen-induced arthritis (AIA) model, IRF-5-deficient mice display reduced knee swelling relative to wild-type mice, owing to a reduction in infiltrating neutrophils in the joint at early stages of disease. Macrophage numbers remain unaffected by loss of IRF-5, but the proportions of MHC II<sup>+</sup> proinflammatory macrophages decrease while the CD206<sup>+</sup> population increases relative to wild-type. Moreover, levels of the neutrophil chemoattractant CXCL1 are reduced in the absence of IRF-5, overall and specifically in synovial macrophages. Ablation of IRF-5 leads to a reduction in macrophage-secreted proinflammatory cytokines and an overall altered inflammatory environment in the joint. Consequently, disease severity is attenuated at later stages of the disease. Both the T-cell response in the knee and histological signs of membrane thickening and bone erosion are diminished in IRF5<sup>-/-</sup> mice.

Table 1 | Selected properties of classically activated (M1) and alternatively activated (M2) polarized macrophages<sup>83-86</sup>.

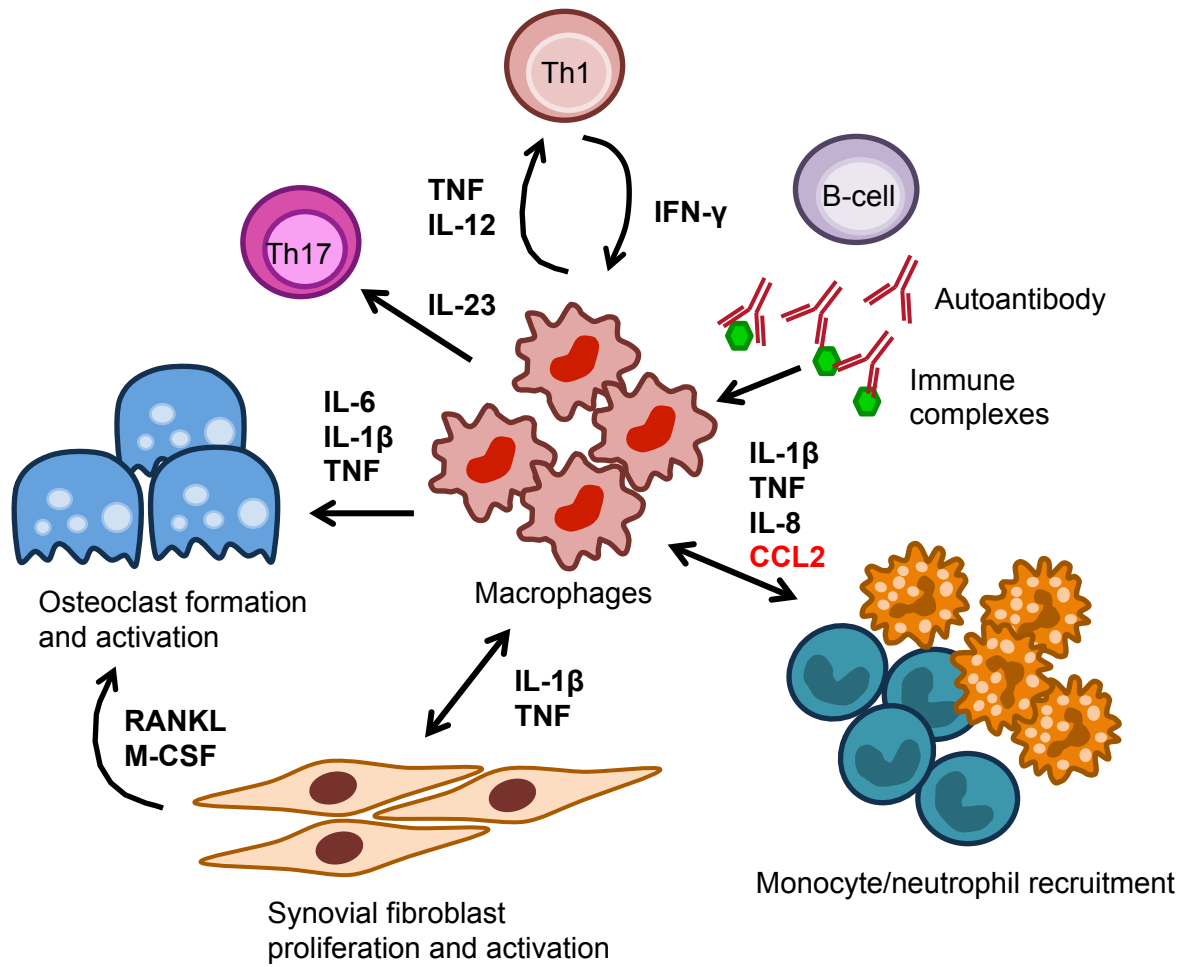
Properties	M1 (interferon $\gamma$ + lipopolysaccharide)	M2 (IL-4/IL-13)
Transcription factors and signalling	Signal transducer and activator of transcription 1 $\alpha$ /B (Stat1) Interferon regulatory factor 5 (IRF-5) Suppressor of cytokine signalling 1 (SOCS1) Nuclear factor $\kappa$ B	Signal transducer and activator of transcription 6 (Stat6) Interferon regulatory factor 4 (IRF-4) Suppressor of cytokine signalling 3 (SOCS3) Krueppel-like factor 4 (KLF4) Peroxisome proliferator-activated receptor $\gamma$ (PPAR- $\gamma$ ) proto-oncogene c-Myc
Cytokines	IL-12, IL-23 TNF, IL-1	IL-10 IL-1 receptor antagonist, IL-1R2
Chemokines	CXCL9, CXCL10, CXCL11, CCL5	CCL17, CCL22
Surface receptors	MHCII high	CD206, mannose, galactose lectin receptor (MGL), stabilin-1 (STAB1), CD163
MicroRNA	miR-29, miR-33, miR-127, miR-155	miR-146a, miR-222, miR-223, let-7c
Metabolism (amino acid)	Inducible nitric oxide synthase (NOS2)	Arginase*
Metabolism (energy)	Glycolysis	Oxidative phosphorylation
Metabolism (iron)	Iron retention	Iron export
Other	NA	Arachidonate 15-lipoxygenase (ALOX15), heme oxygenase 1 (HO-1) Chitinase-like protein 3 (YM-1)*, resistin-like $\alpha$ (Fizz1)*
*Only expressed in mouse cells. NA, not applicable.		

### Author biographies

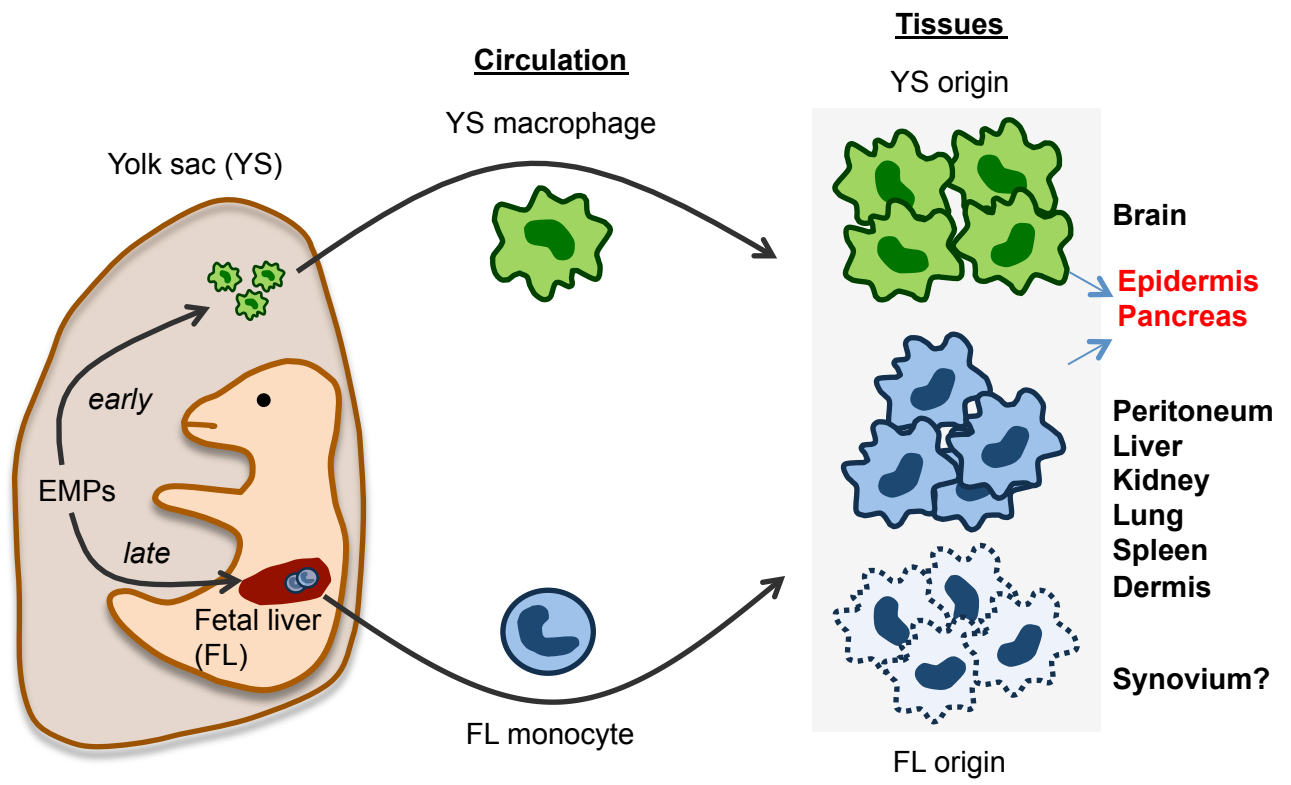
Irina Udalova obtained her BSc/MSc in physics and mathematics and PhD in molecular biology from Moscow Institute of Physics and Technology. She is a Professor of Molecular Immunology at the Kennedy Institute of Rheumatology at the University of Oxford, UK. Her research career began with identification of the role of genetic polymorphisms in the regulatory elements of inflammatory genes, including TNF, and continued with the definition of novel principles of gene regulation by key transcription factor families, and identification of IRF-5 as the master regulator of inflammatory macrophages. Her current research is focused on the genomic and molecular mechanisms controlling inflammatory responses, with specific interests in key molecular switches of myeloid cells.

Alberto Mantovani is an immuno-oncologist with an MD from the University of Milan, Italy, and specialization in Oncology from the University of Pavia, Italy. In the late 1970s he demonstrated the pro-tumour function of tumour-associated macrophages (TAM), linking inflammation and cancer, and he has remained at the forefront of this field of research. He demonstrated the role of chemokines in TAM recruitment, as well as in pathophysiology, including migration of dendritic cells and polarized T cells. His current research and role as Professor of Pathology and Scientific Director of Humanitas Clinical and Research Center, Italy, focuses on exploring the therapeutic potential of targeting TAMs and on understanding the role of pattern-recognition molecules (pentraxins) in innate resistance to infections.

Sir Marc Feldmann is a medically trained immunologist with an MD from the University of Melbourne, Australia, and a PhD in immunology from the Walter and Eliza Hall Institute of Medical Research, Melbourne. He is Emeritus Professor at the Kennedy Institute of Rheumatology at the University of Oxford, UK. He has worked on the role of macrophages in the immune response since the 1970s. His major research contribution is the discovery, together with Sir Ravinder Maini, that TNF is an effective therapeutic target, which has changed the practice of rheumatology, as anti-TNF therapy is extensively used for severe rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis. This discovery has led to a therapeutic revolution, as proteins (chiefly monoclonal antibodies) are now a major sector of therapeutics especially in inflammatory diseases and cancer.



**FIGURE 1:**



**FIGURE 2:**

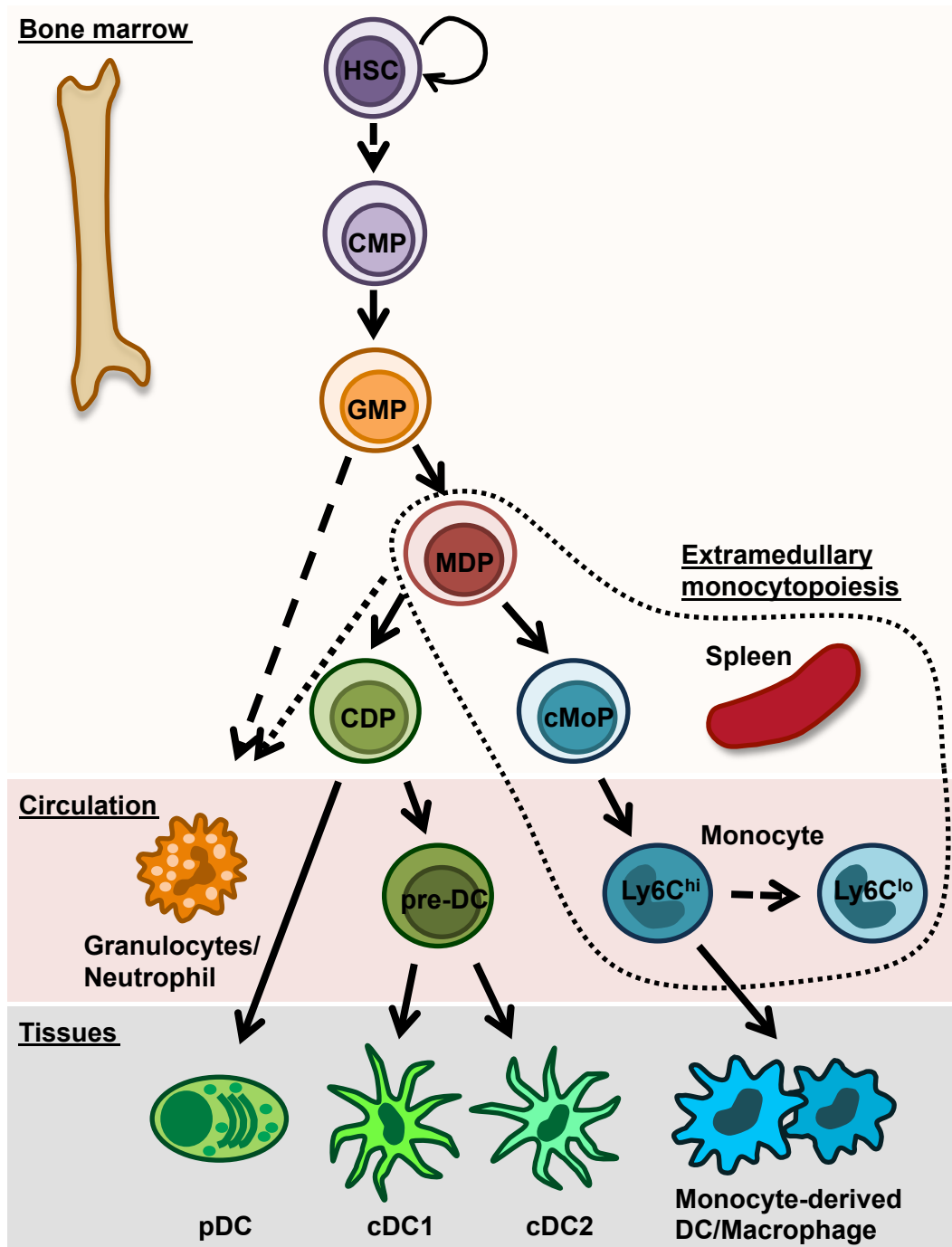
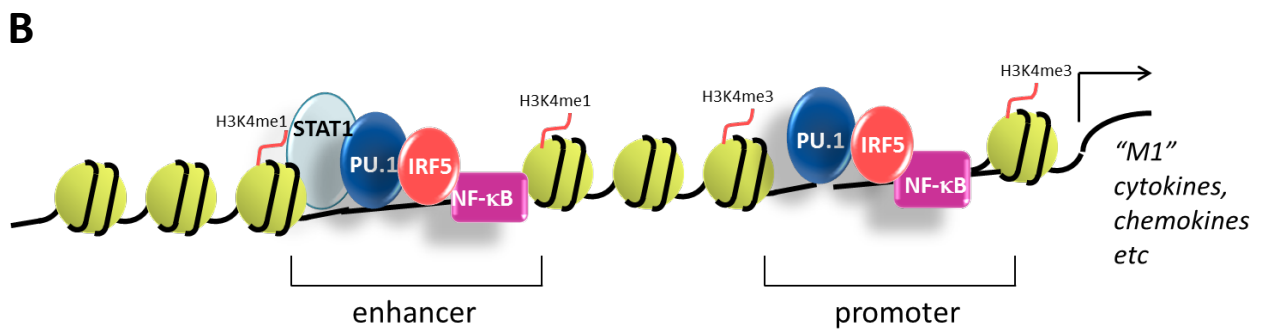
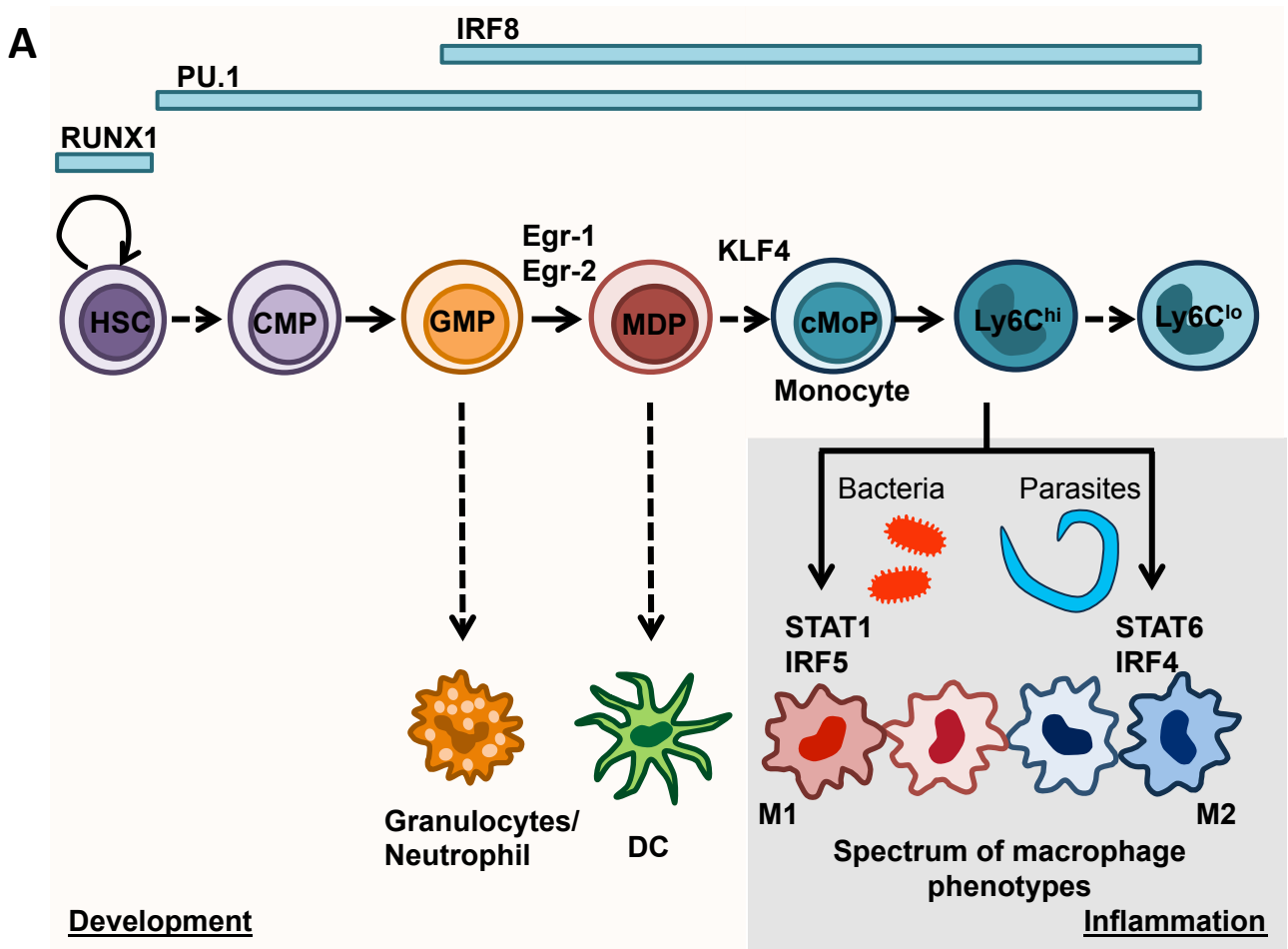
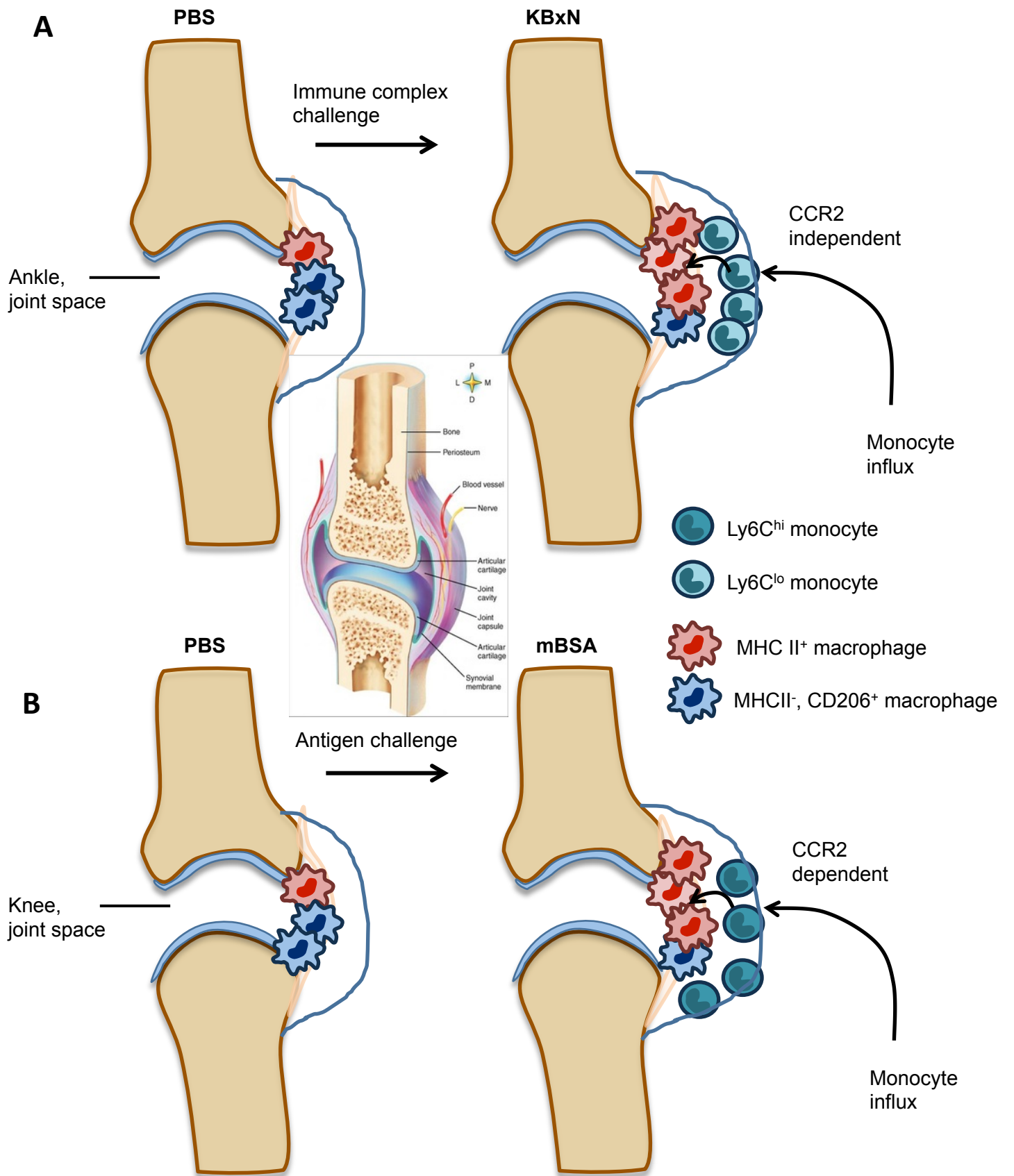


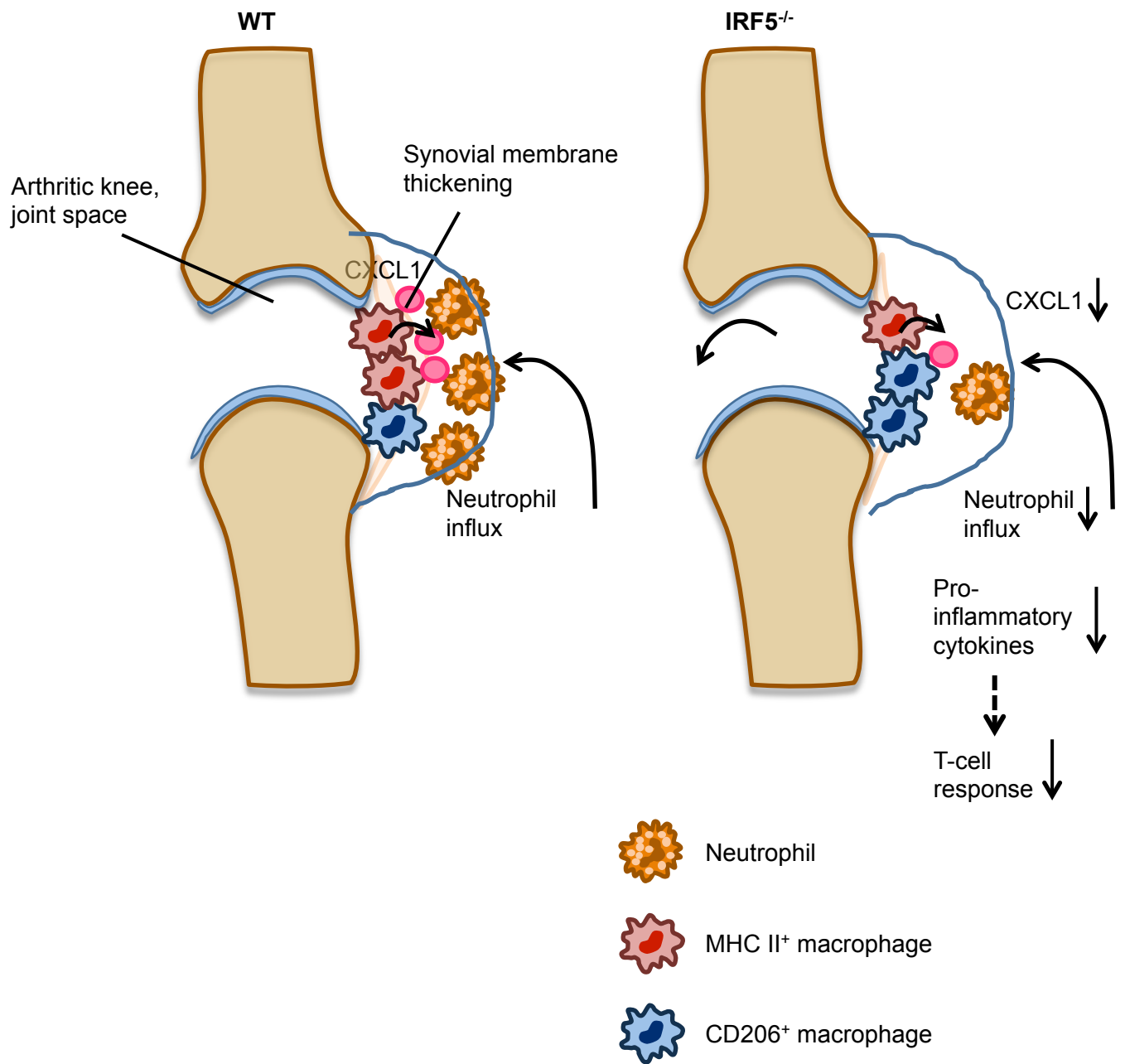
FIGURE 3:



**FIGURE 4:**



**FIGURE 5.**



**FIGURE 6:**