The Dynamic Duo—Inflammatory M1 macrophages and Th17 cells in Rheumatic Diseases

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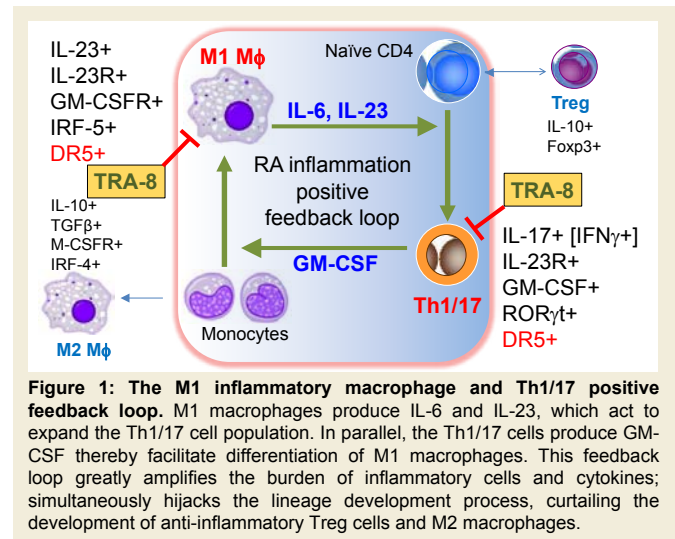
Abstract

The synovial tissue of Rheumatoid Arthritis (RA) patients is enriched with macrophages and T lymphocytes which are two central players in the pathogenesis of RA. Interaction between myeloid cells and T cells are essential for the initiation and progression of the inflammatory processes in the synovium. With the rapid evolution of our understanding of how these two cell types are involved in the regulation of immune responses, RA is emerging as an ideal disease model for investigating the cell-cell interactions and consequently introducing novel biologic agents that are designed to disrupt these processes. This review will discuss the bidirectional interaction between the IL-23^{+} inflammatory macrophages and IL-17^{+} GM-CSF[+] CD4 T cells in rheumatic diseases as well as potential antirheumatic strategies via apoptosis induction in this context.

Introduction

Bidirectional positive feedback loop between macrophages and CD4 T cells

Macrophage subpopulations are developed from monocytes as inflammatory M1 macrophages that produce **Tumor Necrosis Factor-alpha** (TNF-α), **Interleukin** (IL)-6 and IL-23, or anti-inflammatory M2 macrophages that produce IL-10 and **Transformation Growth Factor** (TGF)-β [1,2]. *In vitro* experiments can reproducibly develop such divergent macrophage subpopulations from bone marrow precursors by **Granulocyte-Macrophage Colony-Stimulating Factor** (GM-CSF), **Lipopolysaccharide** (LPS) for M1 macrophages, whereas M2 macrophages, in contrast, can be differentiated with IL-4 or M-CSF. M1 macrophage differentiation is regulated by a transcription factor IRF-5, whereas M2 macrophage is reinforced by the transcription factor IRF-4 [3,4]. CD4 T cell subpopulations likewise can be inflammatory and anti-inflammatory. Inflammatory CD4 T cells include Th1 that primarily produce IFN-γ and Th17 that predominately produce IL-17, but also IL-21, and IL-22. A transitional population of CD4 cells, known as Th1/17, express both IFN-γ and IL-17 and have been found to be the most pathogenic CD4 T cells associated with several disease types including rheumatoid arthritis [5] and **Multiple Sclerosis** (MS) [6,7]. The inhibitory roles of regulatory T cells in RA have also been established in both human patients and mouse models [8].

There is an interdependent and synergistic activity of M1 inflammatory macrophages and Th1/17 inflammatory CD4 T cells (Figure 1). As discussed above, M1 macrophages not only express TNF-α and IL-6, but also IL-23. IL-23 acts through the IL-23 receptor (IL-23R) expressed on CD4 T cells to promote development of Th17 T cells. The primary molecular mechanism for Th17 T cell development is up regulation of the transcription factor, RAR-**Related Orphan Receptor Gamma** (RORγt), after IL-23R stimulation [9-11]. This

Figure 1: The M1 inflammatory macrophage and Th1/17 positive feedback loop. M1 macrophages produce IL-6 and IL-23, which act to expand the Th1/17 cell population. In parallel, the Th1/17 cells produce GM-CSF thereby facilitate differentiation of M1 macrophages. This feedback loop greatly amplifies the burden of inflammatory cells and cytokines; simultaneously hijacks the lineage development process, curtailing the development of anti-inflammatory Treg cells and M2 macrophages.

skews development of CD4 T cells away from an IFN-γ producing Th1 CD4 T cell to an IL-17 producing Th17 CD4 T cell subpopulation. Importantly, the IL-23R-RORγt signaling also promotes the development of the transitional IFN-γ and IL-17 producing CD4 T cell subpopulation, Th1/17 [10], which may be derived from a Th1 population as it undergoes chromatin remodeling. Importantly, GM-CSF marks the highly pathogenic Th1/17 population and can act through the GM-CSF receptor (CSF2R) expressed on macrophages to promote expression of IRF-5 and reinforces the M1 macrophage differentiation. Therefore, the co-existence of IL-23 producing M1 macrophages and GM-CSF producing Th1/17 T cells forms a positive interactive inflammatory positive feedback circuit that is associated with the highest levels of inflammation in autoimmune disease including RA [12], multiple sclerosis (MS) [11], and possibly, **Systemic Lupus Erythematosus** (SLE) [13].

Biologic therapies of rheumatic diseases

Advanced treatment of autoimmune diseases including RA and SLE are currently approached through biologic therapies that target specific pathogenic cytokines or interactive molecules [14]. RA is primarily treated with TNF-α biologic neutralization reagents including soluble receptors to TNF-α [Etanercept (Enbrel‸)] or antibodies to TNF-α [Infliximab (REMICADE‸) and Adalimumab (HUMIRA‸)]. Interactions between **Antigen-Presenting Cells** (APC) and CD4 T cells in RA have been targeted using Abatacept (Orencia), a soluble CTLA4-Ig that blocks the interaction between a co-stimulatory molecule CD28 on T cells with CD86 expressed by antigen-presenting cells [15]. Targeting of CD4 T cells has met with less success [16], however, targeting of IL-17 with an anti-IL-17 antibody may be useful for RA and potentially other autoimmune diseases [17,18]. Anti-B cell therapies for RA and SLE includeanti-CD20 [Rituximab (Rituxan)] or anti-BLyS [Belimumab (Benlysta)], which are out of the scope of the current review.

The advent of biologic agents has led to dramatic changes in the outcomes of therapies for patients with RA and other rheumatic diseases. Specific cell and cytokine targeted therapies have been successfully interrupted the inflammation mediators, disease progression, and led to the impressive advances in our understanding of the immune pathogenesis contributing to the diseases. Inflammatory macrophages and pathogenic Th17 are two critical players in autoimmune diseases. The ultimate goal of effective and safe therapy is to selectively target the central causes of the diseases and reestablish the cellular and functional homeostasis of the immune system and maintain the maximal remission. Therefore, it is conceivable that interference with the vicious cycle between inflammatory macrophages and Th17 may benefit a subset of patients that exhibit inadequate responses to the single target therapy, and might provide an important addition to the current therapeutic paradigms for RA and other rheumatic diseases [5].

Increased death receptor 5 (DR5) expression on inflammatory macrophage and T cell subpopulations: studies of the *viable* motheaten mice

The recessive motheaten (*me/me*) mouse or the allelic viable motheaten (*me^v/me^v*) mouse exhibits mutation(s) of the hematopoietic protein tyrosine phosphatase Src homology region 2 (SHP-2) domain containing phosphatase 1 (SHP-1, PTPN6) [19-21] These mice develop systemic autoimmunity including inflammatory arthritis, splenomegaly, auto antibodies, and renal diseases [22]. The predominant inflammatory cell defect in both motheaten and viable motheaten mice is accumulation of inflammatory macrophages**.** These macrophages exhibited increased proliferative response to GM-CSF but not to M-CSF [23]. We recently showed that the macrophages from the spleen and lymph nodes of *me^v/me^v* mice are predominately IRF-5+ and produce high levels of inflammatory cytokines including IL-23, TNF-α, IL-6 and IFN-γ [5].

Another important feature of the *me^v/me^v* mouse is that DR5, a molecule mediating apoptosis upon binding to its ligand, TRAIL, is up regulated in inflammatory macrophages. However, the DR5^{hi} macrophages are not susceptible to apoptosis *in vivo* [24,25]. In further support of the pathogenic nature of these DR5^{hi} macrophages is that these IRF-5+ macrophages also produce the highest levels of intracellular IL-23 and TNF-α [5]. Therefore, it is likely that IRF-5+ and IL-23+ M1 macrophages in the *me^v/me^v* mouse develop as a result of high levels of GM-CSF, and in part due to an apoptosis defect.

To determine whether depletion of pathogenic macrophages in *me^v/me^v*mice can be achieved by administration of TRA-8, an anti-human DR agonistic antibody, through a novel apoptosis inducing mechanism [26], and whether this can attenuate the inflammation and diseases, we crossed a novel human/mouse (hu/mo) DR5 transgenic (Tg) chimeric mouse with the *me^v/me^v* mouse which develops severe autoimmune diseases and dies prematurely [27]. This Tg mouse expresses the extracellular domain of human DR5 and the intracellular domain of mouse DR5, thereby enabling signaling through the death receptor in a mouse cells after cross linking withTRA-8, a highly-effective anti-human DR5 antibody. Treatment of these DR5 Tg+*me^v/me^v* mice resulted in significant depletion of the IRF-5+, IL-23+, and TNF-α+ subpopulation of macrophages, leading to an increased lifespan of the mice and a significant decrease of Inflammation in lung, kidney, and joints of the mice [5].

A significant population of CD4 T cells in the *me^v/me^v* mouse produced GM-CSF, and the GM-CSF subpopulation of T cells also expressed both IL-17 and IFN-γ, as well as highest levels of Tg DR5 within CD4 T cells [5]. TRA-8treatment led to a dramatic depletion ofTh1/17 (IL-17+IFN-γ+) and Th17 (IL17+IFN-γ-), but less depletion of Th1 (IFN-γ+ IL17-) cells [5].

These results indicated that both of the two cell components in the positive feedback loop, inflammatory macrophages and IL-17+ GM-CSF+ CD 4 T cells, exhibited the highest expression of Tg chimeric DR5, and administration of TRA-8 can overcome the TRAIL apoptosis defect, leading to the depletion of these two highly pathogenic cell populations.

Targeted depletion of M1 inflammatory macrophages down regulates IL-17+IFN-γ+GM-CSF+ CD4 T cells in collagen-induced arthritis (CIA)

To further demonstrate that targeted depletion of M1 inflammatory macrophages can indirectly lead to the decrease of Th1/17 cells, the other component of the circuit, the *Floxed* STOP-hu/mo DR5 Tg mouse was crossed to LysM.Cre mice to enable the Tg chimeric DR5 in macrophages exclusively [27]. The effect of DR5 was analyzed in these LysM. Cre DR5 Tg mice in the context of CIA. TRA-8 treatment resulted in depletion of CD11b+Ly6c+ inflammatory macrophages and significantly reduced development and severity of arthritis. Histologic analysis revealed the reduction of Mac-3+ macrophages with increased apoptosis indicated by TUNEL staining and caspase live imaging in the joints. Cathepsin activity in the joints was also reduced significantly indicated by the ProSense 750 near-infrared fluorescent (NIRF) probes in anti-DR5 treated mice. Histologic examination of joints revealed a significant decrease in osteoclast activity by TRAP staining. To determine if depletion of the inflammatory M1 macrophages has an impact on the CD4 T cell population, FACS analysis of draining lymph node cells revealed that anti-hDR5 treatment depleted Th17 cells, but increased Foxp3+ T regulatory cells, which were also confirmed by real-time PCR analysis in the synovial tissues [27]. These results indicate that selective depletion of M1 macrophage by TRA-8 in a CIA mouse model can indirectly inhibit the development of inflammatory Th17 T cells, and also repopulate synovium and lymph node with Foxp3+ T regulatory cells. These results thus support a model that polarization or maintenance of inflammatory T cells requires M1 inflammatory macrophages and that a cell-based therapy of inflammatory macrophage elimination is an effective strategy to abrogate the inflammatory circuit.
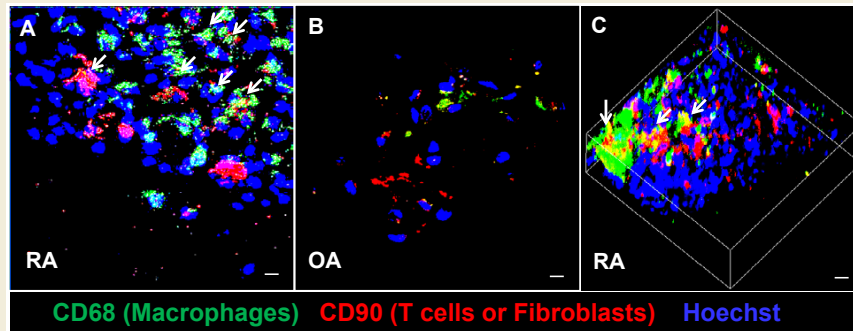
Figure 2: Direct macrophage-T cell interactions in RA synovium. Synovial fragments were isolated from human RA and OA synovial fluid followed by staining with anti-CD68 and anti-CD90 antibodies. Images were then collected using a Zeiss 710 confocal microscope.

(A) RA synovium, 2-Dimension image;

(B) OA synovium, 2-Dimension image;

(C) RA synovium, 3-Dimension reconstruction. Arrow, macrophage-T cell contact. Scale bar, 10μM.

Targeted depletion of Th17 T cells in RA and SLE

We have demonstrated that anti-DR5 can directly induced apoptosis in inflammatory macrophages and indirectly lead to the decrease of Th17 cells in the LysM.Cre DR5 Tg mice with CIA. However, in the me^v/me^v Tg DR5 mice described above, the TgDR5 expression is on both M1 macrophages and T cells. Therefore, the depletion effect of TRA-8 in Th1/17 and Th17 can be potentially direct or indirect. To clarify this, CD4$^+$ T cells were sorted from human RA synovial fluid followed by TRA-8 treatment, which significantly reduced the Th1/17 and Th17 without the presence of M1 macrophages. This result was consistent to the high expression of DR5 in Th1/17 and Th17. Furthermore, it highly indicated that TRA-8 can directly target on pathogenic CD4 T cells in RA [5].

We previously showed that IL-17 producing T cells play an essential role in promoting spontaneous germinal center (GC) development in autoantibody production in the BXD2 mouse model of lupus [28-31]. In this mouse model, development of autoantibody producing GCs was greatly decreased by either blocking of IL-17 with a soluble IL-17R-Fc, or by ablation of IL-17R signaling in B cells using a BXD2-$Il17ra^{-/-}$ mouse [29]. In lupus, the one key pathogenic mechanism is the development of GCs that are essential for development of autoantibody producing B cells which are then pathogenic. The effects of anti-DR5 in blocking pathogenic auto antibodies were evaluated in the hu/mo DR5 Tg Ubc.Cre me^v/me^v mice. In these mice, although the transgene was mainly expressed by inflammatory macrophages and CD4 T cells but not B cells, treatment of anti-hDR5 resulted in a significant decrease in anti-DNA and anti-histone IgM, IgG, IgG1, and IgG2b isotypes and led to a significantly decrease in glomerulonephritis and immune complex deposition of both IgG and IgM "isotypes" "mice". These results together suggest the potential to prevent auto-reactive B cells via modulation of the components in the inflammatory CD4-M1 pathogenic circuit. Consistent with the results we found in RA subjects [5] and previous report [32], we have analyzed peripheral blood mononuclear cells (PBMCs) from patients with SLE and have identified that treatment with TRA-8 resulted in elimination of CD4 T cells, but not CD19 B cells (Hsu et al, unpublished observations). These results suggested that consistent with results obtained from mice and human RA, CD4 T cells, but not B cells from SLE patients could be depleted by TRA-8 therapy.

Analysis of the M1 macrophage Th17 T cell pro-inflammatory circuit in human RA

M1 inflammatory macrophages that produce TNF-α, IL-6 and IL-23 as well as Th17, Th1 have been proposed as key inflammatory players in RA. However, an interactive network between these macrophages and CD4 T cells has been difficult to identify using cells isolated from local inflammatory sites. We first analyzed the gene expression in the whole synovial tissues of RA subjects compared to OA subjects [5]. In RA subjects, there is a significant increase of IL-23, IRF-5 and CSF2RA (GM-CSF receptor) compared to OA, consistent with the increased abundance of M1 macrophages that produce IL-23. In the same synovial tissues, there are also increased levels of IL-17A and CSF2 (GM-CSF) suggesting that an interactive circuit between the M1 macrophages and Th17 T cells that produce GM-CSF could be operative to promote RA inflammation.

In addition to the feedback loop that is mediated by cytokines as described in Figure 1, macrophages-T cells communication can also be fulfilled by direct cell-cell contact. To demonstrate this, fresh synovial fragments were isolated from synovial fluid of RA and OA human subjects. Macrophages and T cells were then visualized by anti-CD68 and anti-CD90 staining (anti-CD90 also recognize human fibroblasts) and analyzed in confocal microscope. As shown in Figure 2A (2 dimensions) and Figure 2C (3 dimensions), the macrophage-T cell interaction is more frequent in RA compared to those in OA (Figure 2B). This observation highly suggested that there is a direct cell-cell contact between macrophages and T cells in RA synovium.

Summary and Conclusions

Autoimmune diseases including RA and SLE exhibit dysregulation and over activation of macrophages, T cells, as well as B cells. Major improvements have been made in the last three decades to target specific mediators of diseases and resulted in significantly improved outcomes in patients. However, certain challenges might still remain prior to the complete revealing of pathogenesis of the diseases. One of the unresolved puzzles is the hierarchy of the pathogenic events in the course of disease onset and progression. Therefore, selectively target two such interacting events, inflammatory macrophages and Th17 cells, simultaneously might exhibit higher efficacy compared to single target, especially when the hierarchy of these two components is not defined within the complexity of immune network.

In this review, we have provided evidence that inflammatory IRF-5^{+} and IL-23^{+} M1 macrophages can potentiate CD4 T cells that produce IL-17 and GM-CSF. Furthermore, these two cell types form an interactive circuit and interruptions of both inflammatory cells simultaneously may provide a more potent therapeutic approach. In both RA and SLE, anti-human DR5 can be used as a model therapy to test this approach. The results show that DR5 primarily targets both the inflammatory M1 macrophages as well as pathogenic CD4 T cells. Furthermore, depletion of M1 macrophages alone indeed can, to some extent, ameliorate the Th17 T cell response, re-enforcing the concept that such a positive feedback circuit does exist. Simultaneously targeting both M1 inflammatory macrophage and Th17 pathogenic CD4 T cells is apotent therapeutic strategy for autoimmune diseases.

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