Review

Cell therapies for refractory rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis (RA), an autoimmune disease, is characterised by a persistent synovitis in the joints and systemic inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs) are widely used to treat RA patients. However, a portion of patients still have inadequate response to traditional medications. Recently, cell-based therapies have become the focus, attracting more attention due to their potential for remission induction. Several immune-regulatory cell types, such as haematopoietic stem cells, mesenchymal stem cells and regulatory T cells have been defined as novel targets. In this paper, we have summarised and reviewed current clinical trials using cell-based therapeutic approaches for the treatment of RA.

Introduction

Rheumatoid arthritis (RA), an autoimmune disease, is characterised by a persistent synovitis in the joints and systemic inflammation. The aetiology of RA is not completely understood. Dysfunction in multiple lymphocytes and excessive releasing of inflammatory cytokines are considered to be involved in the pathogenesis of RA. Currently, RA therapies are mainly dependent on non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs), which are responsible for significant improvements of the symptoms. However, the long-term usage of these drugs can lead to severe adverse effects, including infection, myelosuppression, liver and renal function damage. Moreover, a number of patients still have inadequate response to traditional medications. Therefore, the search for highly efficient and low-toxic therapeutic approaches is critical for the cure of RA. Recently, several studies focusing on stem cell transplantation and targeting inflammatory cells have shown promising results. In this review, we have summarised the current cell-based therapies that are applied for both experimental and clinical treatment of refractory RA.

Haematopoietic stem cells (HSCs)

Characteristic of HSCs

Haematopoietic stem cells (HSCs) are adult stem cells which possess multilineage differentiation and self-renewal potentials, and give rise to all cell types within the blood lineage. HSCs lack specific morphological features. They were identified by virtue of high expression of CD34 and CD90 along with lacking of lineage markers (Lin-)(1). HSCs can be isolated from bone marrow, peripheral blood and umbilical cord blood. They have several unique abilities such as: a) they can self-renewal. HSCs undergo symmetric cell divisions to self-renewal in embryonic phase and perform asymmetric cell division to self-renewal from the late embryonic phase (2); b) they can differentiate into blood cell lineages and non-haematopoietic cells under appropriate conditions (3); c) they have proliferation potentials. Most HSCs stay in the G_0 phase and do not enter the cell cycle. Only a small portion of HSCs are responsible for proliferation at any specific phases (4); d) they possess immune-regulatory properties. HSCs can promote regulatory T cells generation, inhibit auto-reactive T-cells function and reshape the immune system (5-7).

Dysfunction of HSCs in RA

Since the majority of auto-reactive immune cells are the progeny of HSCs, it is suspected that functional defects of HSCs might exist in RA. As expected, RA patients exhibit a low number of bone marrow (BM) $CD34^+$ cells with a defective clonogenic potential (8).

Circulating haematopoietic stem/progenitor cells (HSPCs) are also diminished and displayed a growth factor non-responsiveness independent of age and disease activity (9). Approximately 10–15% of HSPCs exhibit a disability in proliferation, and a delay in the lineage-committed cell differentiation (9, 10). The expression of toll-like receptor (TLR) 3 and IL-1β on HSPCs are high and is associated with inflammatory status in RA patients (11). In addition, RA HSPCs telomere length is shorter than that in age-matched health controls (HCs), indicating that RA HSPCs are susceptible to senescence (9, 10). *In vitro*, BM $CD34^+$ cells from RA patients support spontaneous transformation of peripheral blood B cells from HCs, suggesting that HSCs might contribute to the development of RA via sustaining abnormal B cells response (12). Hence, several experts have proposed that RA is actually "a stem cell disease".

Haematopoietic stem cell transplantation (HSCT) for RA

Due to their immunological properties, haematopoietic stem cell transplantation (HSCT) has emerged as a potential treatment for autoimmune disease. Early animal experimental data demonstrated that autologous HSCT could equally abrogate established arthritis progression and protect against re-challenge (13-16). During the period from 1997 to 2002, it has been reported that several pilot clinical studies use autologous HSCT for refractory RA patients who failed conventional treatment (a mean of 5 DMARDs in total, including a combination treatment). A total of 73 severe RA patients underwent autologous HSCT and were registered in databases of the European Group for Blood and Marrow Transplantation (EBMT) and the Autologous Blood and Marrow Transplant Registry (ABMTR). This retrospective analysis has found significant responses from most of the patients, with over 50% achieving American College of Rheumatology and 50% improvement (ACR50) within 12 months. A significant reduction in the Health Assessment Questionnaire (HAQ) was observed within 18 months. Moreover, the analysis pointed out that rheumatoid factor (RF)-negative patients had a good response to HSCT compared to RF-positive patients. However, the majority of patients suffered from disease flare after 6 months (17). These results indicated that autologous HSCT might be relatively well tolerated and achieve considerably positive responses in the short term. In 2002, another study was performed by comparing the benefits of autologous transplantation of CD34-selected cells *versus* un-manipulated HSCT in prolonging responses for severe RA patients. The initial results showed similar effects and recurrence in patients using CD34-selected cells or un-manipulated transplantation (18). As intrinsic defect of RA HSCs is gradually revealed, which explains why a high frequency of recurrence is followed the autologous HSCT. Thus, allogeneic HSCT is initiated for severe RA. One case report showed that using non-myeloablative allogeneic HSCT to treat an RA patient with a poor prognosis could maintain disease remission for more than 12 months without any additional immunomodulatory medications. No severe infection or graft-versus-host disease (GVHD)was observed (19). The preliminary results indicated that allogeneic HSCT was safe, but further investigation still appeared to be needed.

In summary, clinical evidence indicates that HSCT is relatively well-tolerated and maintains remission in the short-term for severe RA patients. However, patients need to receive a cytotoxic regimen before HSCT and this has potential immunologic complications. The benefit/risk ratio is relatively low. Thus, HSCT has limited therapeutic capacity in rare treatment-resistant patients. More importantly, other stem cells have been found to have an immune-regulatory role without conditioning the regimen before transplantation. This intense approach might be replaced in the future by highly-efficient and low side-effected cell therapies.

Mesenchymal stem cells (MSCs)

Properties of MSCs

Mesenchymal stem cells (MSCs) are adult multipotent stromal cells which are capable of self-renewal and differentiating into different cell lineages including osteoblasts, chondrocytes and adipocytes. They are originally isolated from bone marrow but now they can derive from adipose tissue, umbilical cord, amniotic membrane, placenta and synovium. MSCs express CD73, CD105 and CD90, but lack the haematopoietic and endothelial markers CD34, CD45, CD11b, CD31, CD14, human leukocyte antigens (HLA)-DR. Moreover, MSCs express low levels of major histocompatibility complex (MHC) class I and lack MHC class II, CD40, CD80 or CD86 co-stimulatory molecules. All these features mean that MSCs are less immunogenic (20). Another critical feature of MSCs is the potent immunosuppressive capacity. MSCs inhibit T cell proliferation and activation in response to mitogenic or antigenic stimulation dose-dependently through cell cycle arrest in the G_0/G_1 phase (21, 22). In addition to T cells, MSCs exert inhibitory effects on many other kinds of cells. They are capable of suppressing B cell proliferation and antibody production, reducing cytotoxic activity and cytokine production of natural killer (NK) cells, along with inhibiting antigen-presenting cells (APC) maturation and costimulatory molecules expression (23-27). Besides their immunosuppressive action, MSCs could also induce T cells to exhibit a regulatory phenotype ($CD4^+CD25^+$transcription factorforkhead box protein 3 $(foxp3)^+$ and IL-10-producing T cells) or recruit regulatory T cells (28, 29). Moreover, MSCs could facilitate the polarisation of macrophages towards an M2-like phenotype (27, 30) and change cytokine secretion profile of dendritic cells (DC) to be tolerant phenotype (31). All these MSCs-mediated immune-regulatory effects on both innate and adaptive immunity are through cell-to-cell contact or secreting soluble factors, such as hepatocyte growth factor (HGF), prostaglandin-E2 (PGE2), transforming growth factor (TGF)-β, indoleamine 2,3-dioxygenase (IDO), IL-10 and human leukocyte antigen (HLA)-G (32-35).

The immune-regulatory role of MSCs in RA murine model

As for the ability of differentiation into various cell types, it was initially

considered that MSCs transplantation (MSCT) might repair articular cartilage with their regenerative properties. However, transfusion of MSCs into cartilaginous lesions did not lead to satisfactory regenerated tissue but fibrocartilage formation (36). Luciferase-labelled MSCs that infused into collagen-induced arthritis (CIA) mice intravenously were detected in muscle, lung, spleen, and brain, but not in the joint of MSCs-treated and these infused MSCs disappeared 11 days after treatment, suggesting that the regulation of MSCs in RA was not through differentiation into new tissue but in other ways. As mentioned above, MSCs have been shown to exert immunomodulatory properties on various immune cells *in vitro*. Consistently, in RA, MSCs could also induce T cells hypo-responsiveness and promote $CD4^+foxp3^+$ regulatory T cells expansion (37-39). Fibroblast-like synoviocytes (FLSs) and osteoclast activation have been considered to be involved in the pathogenesis of RA. MSCs were also capable of suppressing FLSs proliferation, invasion and secretion of inflammatory factors (37, 38). Furthermore, a strong inhibitory potential of MSCs on receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclast differentiation was observed (40, 41). Although *in vitro* studies confirmed the suppressive function of MSCs on immune cells, it was still in debate for the therapeutic effects on CIA mice. The first study showed that the allogeneic MSCs did not confer any benefit and even worsened the disease (42), but follow-up reports demonstrated that allogeneic MSCs could reduce the incidence and severity of CIA (38, 43-48). The reason for the difference in efficacy might be related to the infusion time, dose (10^5 *vs*. 10^6), and route (intravenous injection *vs*. intraperitoneal injection *vs*. intraarticular injection *vs*. intralymphatic injection) of administration of MSCs. Besides, only injection of MSCs on Day 18 and 24 improved the arthritic symptoms, suggesting that MSCs demonstrate therapeutic effect during a narrow therapeutic window. The therapeutic mechanism of MSCs in CIA includes: a) to reduce the serum concentration of inflammatory cytokines and chemokines; b) to decrease antigen-specific Th1/Th17expansion and shift Th1/Th2 type responses in lymph nodes and joints; c) to induce antigen-specific $CD4^+CD25^+foxp3^+$ T cells or Tr1 ($IL\text{-}10^+CD4^+$) cells generation. Importantly, it is worth mentioning that modifying MSCs such as engineered to overexpress TGF-β or IL-10 or cytotoxic T lymphocyte antigen (CTLA)-4 or transfected with recombinant minicircles encoding TNF-α blocker could upregulate the effect of naïve MSCs on CIA (49-51). This suggests that enhancing the immunomodulatory activity of MSCs via gene modification might be a gateway for new therapeutic clinical approaches.

Clinical application of MSCT for RA patients

Initially, a brief report showed that RA patients that received autologous BM-MSCs through vein or intraarticular improved clinically and cast off steroid (52). However, RA BM-MSCs exhibited reduced proliferative potential in association with premature telomere length loss and altered gene expression in focal adhesion and cell cycle pathways (53), making allogenic MSCs as a possible way to achieving clinical benefits. Allogeneic MSCs transplantation into four anti-TNF failing active RA patients showed that three out of four patients experienced a reduction in erythrocyte sedimentation rate, disease activity score (DAS) 28, and pain visual analogue scale (VAS) score at the 1st and 6th month after transplantation. Two patients had a European league against rheumatism (EULAR) moderate response at the 6th month but experienced a relapse at the 7th and 23th month, respectively, and two patients had no EULAR response (54). The possible reason why some patients had no response might be that the inflammatory milieu in the RA synovium adversely affected MSC function.

The first cohort study enrolled 173 RA patients who had inadequate responses to traditional medication to assess the safety and efficacy of umbilical cord (UC)-MSCs plus DMARDs. The result demonstrated that both HAQ and DAS28 in the UC-MSCs plus DMARD group showed a steady reduction after 6 months of treatment. The inflammatory cytokine TNF and IL-6 accompanied with RF were significantly decreased, and peripheral blood $CD4^+CD25^+foxp3^+$ regulatory T cells were increased after 3 months in the UC-MSC group. These results indicated that clinical efficacy of MSCs might benefit by their construction of immune balance (55). In a phase Ib/IIa clinical trial, intravenous infusions of allogeneic adipose-derived MSCs (AD-MSCs) into 46 active refractory RA patients were in general well tolerated and clinically beneficial. However, it did not last more than 3 months, suggesting that cell therapy in RA would require repeated administration (56).

Taken together, allogeneic MSCT is a relatively safe and efficacy treatment in refractory RA. With their high ability of immune-regulation and low immunogenicity, MSCs will be more suitable in the clinic for refractory RA patients.

Regulatory T cells (Tregs)

Properties of Tregs

Regulatory T cells (Tregs) are cell population specialised to maintain immunological self-tolerance and homeostasis. The original markers of Tregs are CD25 and foxp3. Afterwards, such marker as CD127, CTLA-4, glucocorticoid-induced TNF receptor family related gene (GITR), lymphocyte activation gene (LAG)-3, CD39 are used for identifying Tregs (57). Tregs modulate immune responses mainly by four basic mechanisms:

a) to inhibit APC maturation and function. Tregs could downregulate the expression of the costimulatory molecules CD80 and CD86 that is necessary for antigen presentation (58). They could also limit APC to initiate an adaptive immune response through interaction between CTLA-4 and CD80/CD86 (59).

b) to induce apoptosis of target cells. By the release of granzymes which enter effector T cells, Tregs could induce effector T cells apoptosis (60-62).

c) to disrupt metabolic pathways. Tregs express ecto-enzymes CD39 and CD73 which enable to catalyse the degradation of adenosine triphosphate into

adenosine (63, 64). Binding of adenosine to its receptor could not only inhibit effector T cells function but also enhance Tregs generation.

d) to secret anti-inflammatory cytokines. Anti-inflammatory cytokines TGF-β, IL-10 and IL-35 released from Tregs are key mediators of Treg function (65-68).

Dysfunction of Tregs in RA

RA is a chronic autoimmune disorder, in which T cells, B cells, DC and osteoclasts are over-activated. Tregs exhibit suppressive ability on these cells, suggesting that they are critical in hampering the development of RA (69-71). However, there is little evidence to show that the number of Tregs is abnormal in RA patients. The ability of RA Tregs to suppress effector T cells proliferation is not impaired either. Nevertheless, they are not able to suppress the proliferation of B cells and production of IFN-γ and TNF-α by effector T cells (72-77). This dysfunction in Tregs is considered to be associated with a lack of CTLA-4 accumulation (78).

Tregs-based therapy for RA

In general, increasing the Treg number or enhancing the suppressive function of Tregs may prove to be beneficial in the suppression of autoimmune diseases, including arthritis. Animal experiment have shown that injection of polyclonal Tregs into CIA mice slowed down the disease progression (79). Depletion of Tregs prior to immunisation or disease presentation led to increased incidence and severity (80, 81). Another animal study found that collagen type II-specific Treg infusion significantly ameliorated arthritis by shifting the Th17/Tregs balance (82). These results suggest that Treg injection benefits RA. Although the therapeutic potential of Tregs is well established in animal models, Treg-based therapy has not been directly applied to RA patients because of several technical challenges. Firstly, no definite surface markers could identify a homogenous Treg population. In fact, activated T cells transiently express CD25, foxp3 and CTLA-4. Transfusing Tregs might be potentially contaminated by effector T cells. Secondly, the expression of foxp3 is instable. A minor population of foxp3$^+$ cells lose foxp3 expression over time, which might become pathogenic (83). *In vitro*, stable foxp3 expression could be induced in the presence of TGF-β. However, TGF-β-induced fox3$^+$ T cells are anergic and produce high levels of effector cytokines (84). Thirdly, Tregs are difficult to expand. In contrast to mice, from which a large number of Tregs can be isolated from spleen and lymph node, human peripheral blood or umbilical cord blood derived Tregs are inherently resistant to expansion. They are susceptible to spontaneous cell death or cytokine-deprivation induced death (85). For these problems, strategies have been created by transfecting ectopic foxp3 or anti-apoptotic gene into T cells. These transfected cells significantly hampered the development of arthritis (86-88). However, whether this technology could apply to the clinic still needs confirmation.

Although it is difficult to expand Tregs *in vitro*, attempts to recover Treg proliferation ability and function *in vivo* are ongoing. IL-2 is essential for Treg maintenance and survival (89). In *in vitro* experiments, Tregs can be expanded by antigenic stimulation in the presence of a high concentration of IL-2 (89). In an *in vivo* system, injection of IL-2 monoclonal antibody into mice resulted in a 10-fold expansion of Tregs (68). Moreover, low-dose IL-2 treatment on graft-versus-host disease (GVHD), hepatitis C virus and cryoglobulinaemic vasculitis patients showed Treg expansion without activating effector T cells (90-92). Thus, IL-2 might show promise in becoming a regulatory factor for Tregs *in vivo*. The other developing drug was rapamycin, which was confirmed to have a positive effect on Treg viability and expansion in GVHD mouse models (93). In kidney transplanted patients receiving a rapamycin-based immunosuppression regimen, an increased proportion of Tregs was also observed (94). Nevertheless, because of the limited reports, whether this drug-mediated Treg upregulation could be used for RA treatment requires further clinical trials.

B cell-targeted therapy

The role of B cells in the pathogenesis of RA

The major role of B cells in RA is the excessive production of antibodies against such self-antigens as RF and anticitrullinated protein antibodies (ACPA), which are well-established indicators of disease progression (95, 96). Except for producing pathologic auto-antibodies, B cells can act as efficient APCs. Upon activation by cognate antigens, B cells process and present antigens to T cells to initiate an immune response (97). In addition, B cells are an important source of inflammatory cytokines. They can produce a wide spectrum of cytokines. An assessing cytokine profile from the synovial fluid of RA patients showed that B cells expressed transcripts for IL-12p35, IL-12p40, IL-23p19, IL-7, IL-15, TNF-α, LT-β, B cell activating factor (BAFF), a proliferation-inducing ligand (APRIL) and RANKL (98). Notably, synovial B cells expressed RANKL, a key cytokine that promoted osteoclasts towards osteoclastogenesis, suggesting a positive role of B cells in bone erosion in RA. A further study showed that plasma cells and B cells were adjacent to activated osteoclasts in RA patients and a significant numeric correlation between plasma cells and osteoclasts was identified (99). Moreover, a cross-sectional cohort of RA patients revealed that the CD5$^+$ B cell population was associated with bone resorption (100). These results support the pathogenic role of B cells in bone destruction.

Anti-CD20 therapy: Rituximab

Rituximab is a chimeric mouse-human monoclonal antibody directed at the CD20 molecule expressed on the surface of human B cells. It is the first drug to target B cells in RA. Since the CD20 antigen is not expressed by pro-B cells or fully differentiated plasma cells (101), rituximab does not prevent the regeneration of CD20-positive B cells from precursor cells or directly interfere with the production of immunoglobulin. The first open-label studies on rituximab in 2001 described a beneficial effect on refractory cases of RA (102). Afterwards, the first randomised double-blind placebo-controlled trial (RCT)

observed that superior ACR20/50/70 responder rates were maintained in the rituximab plus methotrexate (MTX) group after 48 weeks compared with MTX or rituximab monotherapy or rituximab plus intravenous cyclophosphamide (CYC) (103). Similar results were also observed in the following phase IIb dose-ranging trial (DANCER). The DANCER study evaluated the efficacy of MTX plus rituximab for 465 RA patients who had no response to DMARDs. At week 24, a significantly higher proportion of patients achieved ACR20/50/70 and EULAR moderate/good responses in the rituximab plus MTX groups (104). The Phase III RCT REFLEX study showed that more patients achieved the ACR20/50/70 and EULAR moderate/good responses in the rituximab with concomitant MTX group (105). Furthermore, progression of radiological damage was significantly lower in the rituximab with concomitant MTX group after 2 years (106). For RA patients with inadequate response to MTX, good response was also observed in the MTX plus rituximab group in the multicentre phase III RCT study (SERENE) (107). Subsequently, open-label trial (SUNRISE) and multicentre study (RESET) showed the same positive results that patients who had failed anti-TNF achieved significant efficacy after receiving rituximab retreatment (108, 109). Recent data from CERERRA (The European Collaborative Registries for the Evaluation of Rituximab in Rheumatoid Arthritis) collaboration demonstrated that initial treatment with RTX at 500 mg × 2 or 1000 mg × 2 showed comparable clinical outcomes at 6 months, and repeated treatment with rituximab, especially fixed-interval retreatment, led to further clinical improvement than on-flare retreatment (110, 111).

Anti-CD20 therapy: Ofatumumab and Ocrelizumab

Ofatumumab and ocrelizumab are monoclonal antibodies, humanised to reduce immunogenicity, which target extracellular domains of the CD20 antigen. Ofatumumab has been shown to cause selective and prolonged B cell depletion that is mediated via multiple pathways, and to induce potent complement-dependent cytotoxicity and effective antibody-dependent cell-mediated cytotoxicity (112, 113). Phase II/III clinical study results showed that ofatumumab/ocrelizumab treatment achieved ACR response and a good/moderate EULAR response and DAS28 improvement combined with inhibiting joint damage progression in RA patients with inadequate response to MTX, but not in the case of previous anti-TNF failure (114-119). However, positive therapeutic effect was balanced with a high incidence of serious infection (117-119). Therefore, ofatumumab/ocrelizumab has not been licensed for clinical use in RA. To further determine the safety of ofatumumab, a recent study observed the safety of patients who participated in phase II and III trials receiving open-label retreatment. The result showed that serious infections were uncommon and did not increase over time (120). Therefore, whether ofatumumab/ocrelizumab is safe for use still needs more results from RCT studies.

Anti-B lymphocyte stimulator (BLyS) therapy: Belimumab

The B lymphocyte stimulator (BLyS) is a survival factor that binds to specific receptors on B cells. BLyS inhibits apoptosis of B cells and promotes their proliferation and antibody production. BLyS regulates the survival and maturation of B cells through binding with BAFF receptor expressed on the surface of B cells (121). Belimumab is a monoclonal humanised antibody targeting soluble BLyS and prevents BLyS from engaging its receptors on B cells. A Phase II multicentre RCT study evaluated the therapeutic effect of different doses of belimumab combined with DMARDs and NSAIDs and/or prednisone for longstanding moderate-to-severe RA patients. The results showed that ACR20 response was achieved only by 34.7%, 25.4% and 28.2% in the 1, 4, 10 mg/kg group, respectively. However, belimumab failed to improve ACR50/70 responses (122). Another study using belimumab to treat RA involved 283 patients with disease activity despite DMARD therapy. The ACR20 response rate was only 29% 24 weeks after combined DMARDs/belimumab therapy (123). Therefore, more clinical studies are needed to confirm the efficacy of belimumab treatment for RA patients.

Anti-APRIL therapy: Atacicept

APRIL is a homologue of BLyS with biologic functions comparable to BLyS (124). Atacicept is a human recombinant fusion protein that can prevent both BLyS and APRIL from binding to their receptors on B cell. A phase Ib, multicentre, RCT assessed the effect of escalating subcutaneous doses on RF-positive RA patients. The result indicated that although a reduction of RF and anti-CCP antibody was observed, the effect was not significant (125). The following phase II study (AUGUSTI) was carried out to evaluate the therapeutic effect in patients with inadequate response to anti-TNF treatment. The result has also shown a reduction in RF level but not enough to cause a significant improvement in RA patients (126). Similarly, only a modest effect of atacicept was reported on RA patients with no response to MTX (127). Moreover, combination of atacicept with rituximab was not associated with a significant clinical benefit (128). Due to the poor results, ataciceptis has so far not been allowed on the market.

T cell-targeted therapy

CTLA-4 Ig: Abatacept

Abatacept, a fully human fusion protein consisting of the extra-cellular domain of CTLA-4 with the Fc portion of immunoglobulin-G1, has been listed for the treatment for RA. Abatacept selectively modulates the CD28:CD80/86 costimulation signal that is necessary for T cell activation. Hence, abatacept has the capacity to suppress T cell activation and proliferation.

An initial RA murine experiment found inhibition of memory response and decrease in effect or memory populations after abatacept therapy (129). Asubsequent animal study revealed that abatacept restricted antigen-specific T cell proliferation, activation and prevented antigen-specific T cell from acquiring T follicular helper (Tfh) cell phenotype, resulting in reduced specific antibody responses *in vivo* (130). In RA patients,

abatacept suppressed the myeloid dendritic cell-driven activation of both peripheral blood and synovial fluid $CD4^+$ T cells *in vitro* (131). After treatment for 3 and 6 months, abatacept downregulated T cell effector subsets including Th1, Th2 and Th17 (132). Moreover, after 48 weeks of treatment, abatacept induced reduction from baseline in the proportion of circulating $CD8^+CD28^-$ T cells, with this reduction directly correlated with clinical response (133). Recently, a marked decrease in the proportion of Tfh cells was found after abatacept therapy, suggesting more novel T cell subsets could be inhibited by abatacept (134, 135).

Clinically, Phase III study results showed that abatacept treatment obviously improved ACR20/50/70 responder rate together with DAS28 and inhibited radiographic progression in refractory RA patients with an inadequate response to MTX over 6 months, 1 year, 3 years and 5 years (AIM and ACQUIRE) (136-140). For RA patients resistant to TNF-α inhibitors, switching directly to abatacept plus DMARDs exhibited clinical and functional benefits in disease activity and physical function over 6 months, 2 years and 5 years (ATTAIN) (141-143).

Conclusions

The development of RA is accompanied by a breakdown of immune tolerance. Auto-reactive T and B cells are activated, ultimately leading to persistent synovitis and bone destruction. Clinically, there is a proportion of refractory active RA patients, who are resistant to traditional medications. Cell-based therapies, however, due to their ability to target auto-reactive T and B cells, restoring immunological tolerance and re-establishing immune balance, may be an alternative therapeutic option for treatment of RA. Although current clinical data have shown promising effects of cell-based therapies, much more work is still needed to clarify several critical aspects such as the dosage, therapeutic window, combination therapy, long-term effects and side effects, etc. When more data from extensive studies is available, cell-based therapies may be reliably used in the treatment of RA in the future.

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