Mini-Review: The Administration of Apoptotic Cells for Treating Rheumatoid Arthritis: Current Knowledge and Clinical Perspectives

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Rheumatoid arthritis (RA) is a chronic immune-mediated disease managed by conventional synthetic drugs, such as methotrexate (MTX), and targeted drugs including biological agents. Cell-based therapeutic approaches are currently developed in RA, mainly mesenchymal stroma cell-based approaches. Early-stage apoptotic cells possess direct and indirect anti-inflammatory properties. During the elimination of dying cells (a process called efferocytosis), specific mechanisms operate to control immune responses. There are compelling evidences in experimental models of arthritis indicating that apoptotic cell administration may benefit joint inflammation, and may even have therapeutic effects on arthritis. Additionally, it has been demonstrated that apoptotic cells could be administered with standard treatments of RA, such as MTX or TNF inhibitors (TNFi), given even a synergistic response with TNFi. Interestingly, apoptotic cell infusion has been successfully experienced to prevent acute graft-vs.-host disease after hematopoietic cell transplantation in patients with hematologic malignancies, with a good safety profile. In this mini-review, the apoptotic cell-based therapy development in arthritis is discussed, as well as its transfer in the short-term to an innovative treatment for patients with RA. The use of apoptotic cell-derived factors, including secretome or phosphatidylserine-containing liposomes, in RA are also discussed.

Keywords: rheumatoid arthritis, cell-based therapy, apoptotic cells, efferocytosis, inflammation

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic immune-mediated disease that primarily affects the peripheral joints. This inflammatory rheumatic disease is responsible for joint inflammation, cartilage, and bone destruction that progressively lead to joint deformations, structural damage, and disability (1). Rheumatoid arthritis is associated with specific comorbidities, such as an enhanced cardiovascular risk (2), but also systemic complications and premature death (1). Dramatic improvements have been done these past 20 years in the diagnosis and management
of RA. Indeed, new classification criteria have been proposed, and new treatment paradigms together with the development of novel therapeutics have considerably improved the prognosis of the disease (3, 4). Currently, the treatment of RA is based on conventional synthetic drugs [conventional synthetic disease modifying antirheumatic drugs (csDMARDs)] or targeted drugs, including targeted synthetic and biological drugs (tsDMARDs and bDMARDs, respectively) (5). The treatment strategy of RA has been now clarified, and specific international recommendations help the physician in the drug choice and the therapeutic management, taking into account disease activity and severity (6). However, a substantial proportion of patients does not respond adequately to these drugs and, some of them do not achieve remission or low disease activity (7), the therapeutic goals in RA. In addition, the long-term use of these drugs could lead to severe adverse events (8).

Cell-based therapies have been developed in the treatment of RA (9). Administration of cells that can modulate or even dampen the inflammatory process of RA is an exciting therapeutic challenge. Preliminary data with the administration of hematopoietic or mesenchymal stem cells in phase 1/2 clinical trials are currently available in patients with RA (9). In these approaches, the mode of action of hematopoietic or mesenchymal stem cells is still under debate. Administration of apoptotic cells could represent another relevant cell-based therapy for the treatment of RA (10). This cell therapy—developed in different experimental indications, such as transplantation or immune-mediated diseases—has been shown to modulate the immune response and to control, at least partially, the inflammatory process in experimental models of arthritis (10, 11). Thus, in this review, we will comment the current knowledge of apoptotic cell-based treatment in arthritis, and discuss the potential use of this cellular therapy in patients with RA.

RHEUMATOID ARTHRITIS: A BRIEF OVERVIEW OF ITS PATHOPHYSIOLOGY

The exact cause of RA remains currently unknown. Its development results from the complex interplay between genetic, environmental, hormonal, and psychological factors (12). Rheumatoid arthritis is characterized by inflammation and hyperplasia of the synovium, leading to the so-called rheumatoid “pannus.” Joint inflammation in RA is related to an immune activation with the participation of both innate immune cells [i.e., neutrophils, monocytes and macrophages, NK cells, dendritic cells (DC), mast cells, and innate lymphoid cells], and adaptive immune cells (Th1 and Th17 cells, B cells, and plasma cells). A strong cellular response has been initiated within the synovium with the activation of local synovial cells, including fibroblast- and macrophage-like synoviocytes (13). These resident synovial cells exhibit an aggressive phenotype with the production, together with immune cells, of a complex network of soluble factors, i.e., cytokines and chemokines, perpetuating the cellular activation and inflammatory reaction (14). Resident synoviocytes also produce metalloproteases that degrade the extra-cellular matrix components, i.e., cartilage and subchondral bone, ultimately promoting joint destruction (15). Rheumatoid arthritis is characterized by the production of well-described autoantibodies, of which rheumatoid factors (RF) and anti-citrullinated protein antibodies (ACPA) are the most common (16). ACPA can bind citrullinated motifs expressed by several self-proteins, including vimentin, fibrinogen, fibronectin, α-enolase, or type II collagen. The presence of RF and/or ACPA is associated with a more severe disease. Post-translational protein modifications explain the reaction of citrullination under the influence of the peptidyl-arginine deiminase enzymatic activity, with subsequent immunization and the production of ACPA (17). Other post-translational reactions have been described in RA, such as the formation of carbamylated residues promoting the synthesis of anti-carbamylated antibodies (18).

The treatment of RA is now well-defined, and has profoundly changed over the past 20 years (4). Methotrexate (MTX) is used as first line csDMARDs, and is considered as the reference treatment in RA (19). Targeted drugs (such as tsDMARDs or bDMARDs) are proposed as second line therapy in the case of inadequate response to csDMARDs (20). Among bDMARDs, TNFi have been available on the market for more than 20 years, and are widely used in clinical practice. Other bDMARDs include IL-6 blocking agents, a T cell costimulation blocker (abatacept) and a B cell depleting agent (rituximab) (21). Janus kinase inhibitors (tofacitinib, baricitinib, upadacitinib and filgotinib) correspond to tsDMARDs. These agents interfere with signal transduction involved in T cell activation (22).

CELL-BASED THERAPIES IN RHEUMATOID ARTHRITIS

Cell-based therapeutic approaches have been recently developed in RA. The rationale is based on the administration of cells exhibiting direct or indirect anti-inflammatory properties targeting inflammatory cells involved in the RA pathophysiology (9). The cells used in RA are mainly stem cells from the hematopoietic or the mesenchymal lineage. Hematopoietic stem cells (HSC) possess the property of multilineage differentiation, resulting in all blood cell types. They are isolated from bone marrow, peripheral blood after G-CSF mobilization, or umbilical cord. The curative effect of hematopoietic cell transplantation (HCT) is based on the conditioning regimen that eliminates pathogenic immune cells and the re-establishment of the host immune system. This procedure has been performed in a limited number of RA patients [reviewed in (9)]. In this setting, allogeneic HCT is tolerated and can improve disease activity and/or maintain remission for the short term. However, these patients received a cytotoxic regimen before transplantation with potential immunologic or infectious complications, and are at risk of developing severe acute graft-vs.-host disease (GvHD).

Another approach is the administration of mesenchymal stem/stroma cells (MSC). These cells are multipotent stromal cells that can differentiate into different cellular lineages. They derive from bone marrow, adipose tissue, umbilical cord, or other organs including the synovium. They exhibit several
immunomodulatory effects, including: inhibition of T cell activation/proliferation, suppression of B cell activation and antibody production, reduction of NK cell activity, generation of regulatory T (Treg) cells, and polarization of macrophages toward an anti-inflammatory phenotype (9). In a large cohort of patients who had inadequate response to csDMARDs, MSCs were administrated intravenously together with csDMARDs and compared to patients who received only csDMARDs. A significant improvement of RA was observed in the MSC group, with a remission sustained up to 3–6 months (23). Another phase 1/2 clinical trial evaluated the safety and efficacy of allogeneic expanded adipose tissue-derived MSCs administered intravenously. That dose escalation, placebo controlled study consisted of 3 infusions over 2 weeks. The treatment was well-tolerated and yielded clinical improvement after 1 month. However, the efficacy was not sustained and the clinical response was mitigated at month 3 (24). Other cellular therapies have been tried in patients with RA, including the intra-articular (IA) administration of autologous tolerogenic DC, leading to promising results (25).

THE IMMUNOMODULARY PROPERTIES OF APOPTOTIC CELLS

Apoptosis is a physiological cell death process that occurs during the development, and throughout the life in all tissues. Every day, a billion of cells die and are eliminated without deleterious consequences as inflammation or inadequate immune response (26). Apoptosis is followed by the efficient clearance of dead cells by professional phagocytes, a mechanism called efferocytosis. Efferocytosis is a key mechanism for tissue homeostasis (26). The appropriate efferocytosis of apoptotic neutrophils is a major event in the resolution of inflammation (27). Conversely, a defective efferocytosis may contribute to impaired tissue homeostasis, delayed tissue repair, and non-resolving inflammation. In this context, inadequate clearance of apoptotic cells may promote auto-inflammatory and/or autoimmune diseases (28). Altered efferocytosis has been reported in RA (28, 29).

Apoptotic cells display immunomodulatory properties that can be harnessed for therapeutic use (10, 11). Indeed, both apoptotic cells themselves and the interaction of apoptotic cells with professional phagocytes are associated with immune regulation mechanisms (Figures 1A–C). Before being removed, apoptotic cells create a local transient suppressive microenvironment (30–34) (Figure 1A). In response to specific signals released by apoptotic cells, called “find me” signals, professional phagocytes move toward the sites where cells are dying (37–39). Professional phagocytes correspond mainly to tissue-resident and monocyte-derived macrophages, as well as specific DC subsets (10). Apoptotic cell engulfment is then driven by specific signals expressed on apoptotic cells or fragments of apoptotic cells [collectively called “don’t eat me” and “eat me” (i.e., phosphatidylserine; Ptdser) signals] (40). While “don’t eat me” signals prevent the uptake of viable cells, “eat me” signals stimulate the engulfment and modulate the immune responses. Thus, liposomes expressing Ptdser have been shown to mimic some immunomodulatory effects of apoptotic cells (41). Apoptotic cells may cooperate with macrophages to induce an immunosuppressive environment (Figure 1B) (35). After efferocytosis, an anti-inflammatory reprogramming of macrophages is initiated (26, 27), leading to a sustained immunosuppressive environment (Figure 1C) through the release of several anti-inflammatory cytokines [e.g., TGF-β, IL-10 (42, 43)], and other regulatory factors (44, 45), such as pro-resolving mediators (10, 11, 46). Reprogramming of macrophages also includes a decreased capacity to produce pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-12, or IL-23) (10). Phagocytic dendritic cells are also maintained in an immature stage and/or developed a tolerogenic profile, contributing to the generation of regulatory immune cells, including IL-10-producing T cells, CD4+ Treg, CD8+ Treg or regulatory B (Breg) cells (Figure 1C). This tolerogenic microenvironment participates in the inhibition of Th1 and Th17 responses (10, 11). Overall, the administration of apoptotic cells may control an ongoing pro-inflammatory process by different regulatory mechanisms and by inhibiting pathogenic cells. All these mechanisms support the rationale to use apoptotic cell administration in chronic systemic inflammation, like in arthritis (46). We thus reviewed the effects of apoptotic cells, apoptotic cell-derived factors, approaches mimicking apoptotic cell injection, or factors released during efferocytosis in animal models of arthritis. A systematic literature search was performed in PUBMED Medline database until November 2020, using the following keywords: [apoptotic cells] OR [apoptotic cell factors] OR [efferocytosis factors] AND [arthritis] AND [animal models]. After title and abstracts selection, duplicate elimination and manual search, 10 articles were finally selected for analysis (34, 47–55) (Table 1).

THE EFFECTS OF APOPTOTIC CELLS IN PRECLINICAL MODEL OF ARTHRITIS

Six articles were selected (47–52). Apoptotic cell-based therapies have been evaluated through intravenous (IV), intraperitoneal (IP), or IA administration of early stage apoptotic cells in the main arthritis models. The main results are summarized in Table 1. According to the experimental model used and to the time of apoptotic cell injection (i.e., at the time of arthritis induction, before clinical symptom occurrence, or during ongoing arthritis), one may distinguish preventive vs. curative effects. A preventive effect of apoptotic cell administration on clinical scores has been observed in all the arthritis models tested (47–50), except in the serum transfer-induced arthritis (STIA) model (47). This negative result may be explained by the experimental model used, lacking the adaptive immune response (46). Indeed, implication of IL-10-producing T cells [T regulatory 1 (Tr1) cells], Treg and/or IL-10-secreting Breg cells has been shown to be triggered by apoptotic cell infusion in arthritis models and associated with the reduction of joint inflammation (47–49) (Table 1). In streptococcal cell wall (SCW)-induced arthritis, IP injection of apoptotic cells along
FIGURE 1 | The immunomodulatory effects of apoptotic cells. (A) Apoptotic cells exert direct anti-inflammatory effects by: (i) neutralizing inflammatory chemokines, via CCR5 expression (30), and (ii) releasing different factors, including: anti-inflammatory cytokines [e.g., TGF-β (31)], mediators [i.e., annexin-A1 (32) or thrombospondin-1 (33)], as well as cellular metabolites [e.g., spermidine, guanosine monophosphate, inosine monophosphate (34)]. (B) Cooperation between apoptotic cells and their phagocytes may mediate immunosuppression. Apoptotic cells release adenosine monophosphate (AMP) which is then converted into adenosine by phagocytizing macrophages. This factor interacts with macrophage adenosine A2A receptors and stimulates anti-inflammatory gene expression (35). (C) Different phagocytes propagate the immunomodulatory effects of apoptotic cells after efferocytosis. Professional phagocytes (immature cDC and macrophages), as well as amateur phagocytes (e.g., fibroblasts, epithelial cells) may participate to efferocytosis (10). The consequences for phagocytes differ depending on the considered phagocytes. Efferocytosis by cDC blocks them in an immature stage or induces a tolerogenic profile that generates regulatory adaptive immune cells: IL-10-secreting cells, CD4+ Treg, CD8+ Treg, and/or IL-10-producing B cells. After efferocytosis, macrophages secrete anti-inflammatory factors (e.g., TGF-β, IL-10, IL-1RA, and HGF) and reduce their production of inflammatory cytokines (e.g., TNF-α, IL-1β, IL-12, and IL-23). Efferocytosis triggers macrophage reprogramming toward a pro-resolving profile (26, 27). Moreover, macrophages may stimulate via extracellular vesicles factors and IGF-1 the efferocytic capacity of amateur phagocytes (36). Finally, these amateur phagocytes may secrete immunosuppressive cytokines, such as TGF-β or IL-10. A2A, Adenosine 2A receptor; AMP, adenosine-monophosphate; Breg, regulatory B cells; cDC, conventional dendritic cell; DHAP, dihydroxyacetone phosphate; FBP, fructose-1,6-biphosphate; GMP, guanosine-5′-monophosphate; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor; IL-, interleukin; IMP, inosine-5′-monophosphate; Mφ, macrophage; PtdSer, phosphatidylserine; TGF-β, transforming growth factor-beta; Treg, regulatory T cells. This figure was depicted, in part, by using Servier Medical Art, https://smart.servier.com/.

with arthritis induction induced a significant improvement of joint inflammation and limited joint destruction (48). In this rat model, the results are more impressive on the chronic stage of the disease (48). A therapeutic effect of IV apoptotic cell administration has been reported in ongoing arthritis using the collagen-induced arthritis (CIA) model (51). In this model, co-administration of MTX with apoptotic cells induces arthritis reduction that was similar to apoptotic cell injection alone, thus demonstrating that MTX does not interfere with apoptotic cell infusion (51). The concomitant administration of a TNFi together with apoptotic cells induces better arthritis reduction (51), indicating a synergistic effect between the mode of action of both therapies. Finally, IA apoptotic cell injection has exerted also a therapeutic effect in a mouse Lyme arthritis model (52). Altogether, these different experimental results well illustrate the beneficial consequences of apoptotic cell administration in acute and chronic arthritis, with both preventive and curative effects. Apoptotic cell-based therapy may thus be potentially able to control ongoing clinical arthritis, to dampen inflammation and attenuate destructive articular changes. This strongly supports the therapeutic use of apoptotic cells in patients with RA.
Four articles were selected for this review (34, 53–55) (Table 1). It has been shown that the intramuscular (IM) administration of Ptdser-containing liposomes in adjuvant arthritis (AA) is able to inhibit AA-induced trabecular bone loss (53). The same group demonstrated that this approach induced macrophage reprogramming with the reduction of IL-1β release and an increase in IL-10 production (54). This confirms that Ptdser-containing liposomes can mimic some effects of apoptotic cells (41). Recently, the administration of a mixture of 3 or 6 metabolites identified in apoptotic cell secretome has been shown to diminish paw swelling in the STIA model (34) (Table 1). These apoptotic cell-derived metabolites are able to control macrophage reprogramming toward a pro-resolving profile (34). Thus, selective apoptotic cell-derived factors may also be considered as innovative therapeutic approaches in arthritis. Furthermore, the secretome of macrophages eliminating apoptotic leukocytes has been shown to exert a therapeutic effect on arthritic clinical score in CIA by inducing collagen-specific CD4+ Treg cells and macrophages toward a tolerogenic state (55). This treatment of

Table 1

| Reference | Experimental model of arthritis | Apoptotic cell source or apoptotic cell mimetic | Route of administration | Main effects | Final result and duration of the effect |
|-----------|---------------------------------|-----------------------------------------------|------------------------|-------------|---------------------------------------|
| (47)      | Collagen-induced arthritis (DBA/1 mice) | Syngeneic early apoptotic thymocytes | IV or IP | Reduction of collagen auto-antibodies; induction of IL-10-producing CD4+ T cells; reduction of IFN-γ secreting CD4+ T cells; IL-10-producing marginal zone B cells | Prophylactic effect on arthritis |
| (51)      | Collagen-induced arthritis (DBA/1 mice) | Syngeneic early apoptotic thymocytes | IV | Reduction of collagen auto-antibodies; induction of collagen-specific Treg cells; resistance of APC to TLR ligand activation, indicating APC reprogramming; effects dependent on TGF-β; synergic results with TNFI | Transient therapeutic effect on arthritis |
| (55)      | Collagen-induced arthritis (DBA/1 mice) | Secretome of macrophages eliminating apoptotic cells | IV or IP | Generation of collagen-specific Treg cells; pro-tolerogenic reprogramming of pDC and macrophages; mediated at least in part by TGF-β | Therapeutic effect on arthritis up to 60 days following secretion administration |
| (49)      | Methylated BSA-induced arthritis (C57BL/6 mice) | Syngeneic early apoptotic thymocytes | IV | Suppression of Th17 response in the draining lymph nodes; increase of IL-10-producing T cells; IL-10-producing marginal zone B cells; effects dependent on natural IgM | Prophylactic effect on arthritis |
| (50)      | Methylated BSA-induced arthritis (C57BL/6 mice) | Syngeneic apoptotic dendritic cells | IV | The use of activated apoptotic cells induces IL-6 and prevents TGF-β-mediated prevention of arthritis | Prophylactic effect on arthritis |
| (47)      | Serum transfer-induced arthritis (C57BL/6 mice) | Syngeneic early apoptotic thymocytes | IV or IP | No prophylactic effect | No improvement of arthritis |
| (34)      | Serum transfer-induced arthritis (C57BL/6 mice) | Apoptotic cell-derived metabolites* | IP | Diminution of paw swelling | Prophylactic effect on arthritis |
| (52)      | Lyme arthritis (C3H/HeJ mice infected with Borrelia burgdorferi) | Xenogeneic Jurkat leukemic cells | IA | A trend toward higher IL-10 levels in the joints | Therapeutic effect on arthritis (accelerated disease resolution) |
| (53)      | Adjuvant arthritis (Lewis rat) | Phosphatidylserine-containing liposomes | IM | Reduction of RANK and RANKL mRNA; inhibitory effect on osteoclast formation; increased production of TGF-β and PGE2 | Therapeutic effect on arthritis (reduction of paw volume at day 28, then rat sacrifice) |
| (54)      | Adjuvant arthritis (Lewis rat) | Phosphatidylserine-containing liposomes | IM | Reduction of RANK and RANKL mRNA; Increased IL-10 and decreased IL-1β and IL-17 in the ankle joint; shift from IL-1β-to IL-10-producing infiltrated macrophages | Therapeutic effect on arthritis (reduction of paw volume at day 28, then rat sacrifice) |
| (48)      | Streptococcal wall-induced arthritis (Lewis rat) | Xenogeneic early apoptotic thymocytes | IP | Decrease in the pro-inflammatory response of peritoneal macrophages; induction of Treg cells in the draining lymph node; effects dependent on TGF-β | Prophylactic effect on arthritis |

APC, antigen presenting cells; BSA, bovine serum albumin; IA, intra-articular ankle injection; IM, intramuscular (in the hind limbs); IP, intraperitoneal; IV, intravenous; pDC, plasmacytoid dendritic cell; PGE2, prostaglandin-E2; TNFI, TNF inhibitors; Treg, CD4+ FoxP3+ regulatory T cells.

*Two cocktails were tested: one with six metabolites [dihydroxyacetone phosphate (DHAP), fructose-1,6-biphosphate (FBP), guanosine-5′-monophosphate (GMP), inosine-5′-monophosphate (IMP), spermidine, UDP-glucose] and the other with 3 metabolites (GMP; IMP; spermidine).
Arthritis has been associated with a restored functional resolution of inflammation (47). This confirms the main role of efferocytosis in the control of inflammation (27), as well as the importance of the macrophage/pDC/Treg cell crosstalk in apoptotic cell-induced tolerance (56). Overall, apoptotic cell “derivatives” can be an alternative to apoptotic cell-based therapy.

**APOPOTIC CELLS IN THE TREATMENT OF ARTHRITIS: TOWARD A CLINICAL USE**

Results from experimental studies detailed above support the rational for a clinical perspective in RA. This is based on the preclinical data, but also on an extensive research in the fields of cancer and transplantation (10, 11, 46). However, there are several concerns before translating these results to a clinical trial in humans. First, in most of the experimental models used to evaluate the apoptotic cell-based approach, arthritis is induced by a known and specific auto-antigen, which is not the case in patients. Second, certain models are self-resolving, without a chronic phase after the acute peak of the disease. This does not mimic the clinical situation. Third, most of the data have been obtained with a therapy given at the initiation of the disease, even before the onset of arthritic clinical symptoms. This is not feasible in clinic. Despite the fact that a mouse is not human and that regulatory mechanisms may differ between these two species (57), the feasibility must be discussed. What type of cell has to be used as apoptotic cells? Indeed, peripheral blood leukocytes are the easiest cell source to consider in human, contrary to apoptotic cells used in experimental models that are from the thymus, the spleen, DCs, and even leukemic cells (Table 1). As observed in experimental settings, apoptotic leukocytes would need to be at an early apoptotic stage, i.e., annexin-V⁺, 7-aminoactinomycin D or propidium iodide negative. These cells may be collected using cytopheresis. Different procedures may render the cells apoptotic and the different stimuli include γ-, X-ray, or UV-irradiation, Fas-mediated cell death, as well as corticosteroid treatment. The appropriate route of administration for the patients is the IV route, since this approach seems appropriate for a systemic disease, such as RA. This allows a quick diffusion of apoptotic cells through the body. To summarize, one may propose the following procedure for the apoptotic cell preparation in a clinical trial: a cytopheresis collecting peripheral blood leukocytes that are then exposed to a 35-Gy dose of γ- or X-ray irradiation to induce apoptosis. In these irradiation settings, one may obtain around 40–50% of cells in an early apoptotic stage, with a limited number of cells (<10%) in a necrotic stage. The concomitant use of csDMARDs (such as MTX) and of bDMARDs (TNFi) is not a limit, since MTX does not alter the therapeutic effects of apoptotic cells (51), whereas TNFi displays a synergistic effect (51). Glucocorticoids (GCs) are still used in clinical practice for the treatment of RA, and this medication may be administered with apoptotic cells. Indeed, GCs enhance apoptotic cell removal during the different phases of apoptotic cell clearance (40). The safety of apoptotic cell infusion is another concern. This cell-based therapy has been previously used in a clinical trial in the setting of HCT (58). In this phase 1/2a trial, donor early apoptotic cells have been administered intravenously the day before allogeneic HCT in order to prevent acute GvHD in 13 patients with hematological malignancies. The administered cells have been obtained from hematopoietic cell donors after cytopheresis. This cell-based therapy has been well-tolerated without related serious adverse events. The overall incidence of stage II to IV GvHD was 25% while in the group of patients who received the highest dose of apoptotic cells, this rate was 0% (58). The conclusion of this trial was that apoptotic cell infusion during HCT is a safe procedure and potentially effective in the prevention of GvHD. This illustrates the feasibility of this cell therapy approach. Considering these results, we plan to start a clinical trial on apoptotic cell infusion in RA patients who do not respond adequately to at least one bDMARD. The APO-RA project is a phase 1/2 trial that proposes one infusion of peripheral blood leukocytes rendered apoptotic cells by X-ray irradiation (ClinicalTrials.gov identifier: NCT02903212).

**CONCLUSION AND PERSPECTIVES**

The use of dying cell or their byproducts as a therapeutic approach in the treatment of RA, or other forms of arthritis, still represents an exciting and innovative therapeutic opportunity. Besides cell-targeted therapies that are currently used in RA (e.g., abatacept or rituximab), and besides MSC or tolerogenic DC administration, apoptotic cells represent another cellular approach with an attractive mode of action. There are compelling evidences demonstrating that apoptotic cell administration may dampen the inflammatory process of RA. Preliminary data in the setting of HCT indicate the feasibility of this approach with a good safety profile. It is, thus, now time to translate all these preclinical data in clinical settings. Some questions persist such as the exact therapeutic protocol to use (number of cells to administer, stimulus to generate early apoptotic cells without high a rate of necrotic cells), and whether a single infusion of apoptotic cells will give a sustained response.

**AUTHOR CONTRIBUTIONS**

ET performed the bibliographic search, wrote the first version of the manuscript, and made the table. PS completed the bibliographic search, edited the first version of the manuscript, and made the figure. All authors reviewed and edited the manuscript.

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Conflict of Interest: FB and SP are employees by MED’INN’PHARMA, which develops a pro-resolutive drug candidate called SuperMapo®. FB, SP, and PS are shareholder of MED’INN’PHARMA.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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