Gene Therapy in Rheumatoid Arthritis: Strategies to Select Therapeutic Genes

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Gene Therapy in Rheumatoid Arthritis: Strategies to Select Therapeutic Genes

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Abstract
Significant advances have been achieved in recent years to ameliorate rheumatoid arthritis (RA) in animal models using gene therapy approaches rather than biological treatments. Although biological agents serve as antirheumatic drugs with suppressing proinflammatory cytokine activities, they are usually accompanied by systemic immune suppression resulting from continuous or high systemic dose injections of biological agents. Therefore, gene transfer approaches have opened an interesting perspective to deliver one or multiple genes in a target-specific or inducible manner for the sustained intra-articular expression of therapeutic products. Accordingly, many studies have focused on gene transferring methods in animal models by using one of the available approaches. In this study, the important strategies used to select effective genes for RA gene therapy have been outlined. Given the work done in this field, the future looks bright for gene therapy as a new method in the clinical treatment of autoimmune diseases such as RA, and by ongoing efforts in this field, we hope to achieve feasible, safe, and effective treatment methods.

1 INTRODUCTION
Rheumatoid arthritis (RA) is a debilitating, chronic intra-articular inflammatory autoimmune disease with no known initiating stimulus that mainly affects joints and leads to synovial hyperplasia, joint damage, and systemic complications in severe cases (Fabre & Apparailly, 2011; van de Loo, Smeets, & van den Berg, 2002). All treatment methods used in the therapy of RA can be classified into two main types of old and novel RA medications. The limitations and benefits of both methods have been thoroughly investigated (Kahlenberg & Fox, 2011; Wilsdon & Hill, 2017). The primary strategy for treating RA is to reduce articular swelling, relieve pain, and control disease progression with the old method drugs (Traister & Hirsch, 2008). These methods can be divided into two main classes including: (1) steroid hormones (nonsteroidal anti-inflammatory drugs and glucocorticoids, and (2) biological agents, disease-modifying antirheumatic drugs that can be divided into two subgroups 2-1: monoclonal antibodies and 2-2: small molecules (Matteson, 2000). Although these drugs are more effective based on the improvement criteria set by American College of Rheumatology, it should be considered that application of these drugs is uncertain because of the existence and the occurrence of some serious side effects. (Burmester & Pope, 2017). Therefore, the development of alternative biotechnological methods such as gene and mesenchymal stem cell therapies have been recognized, respectively, to remove the inherent barriers associated with the effective delivery of therapeutic proteins and to
profit from the immunomodulatory and regenerative properties of mesenchymal stem cells in the treatment of RA (Abdolmohammadi et al., 2019; Fabre & Apparailly, 2011). Progress in the better understanding of RA pathophysiology has led to the development of new biological therapeutic targets, which allow disease modification by targeting immunological mediators as the main source of many side effects of the disease (Adriaansen, Vervoordeldonk, & Tak, 2006). The new biological therapeutic methods can be divided into several classes including treatment of bisphosphonates (Breuil & Euller-Ziegler, 2006), oral tolerance (Toussirot, 2002), nanotechnology, photodynamic therapy (Lu et al., 2018), stem cell therapy (Snowden, Kapoor, & Wilson, 2008), and gene therapy (Table 1). The ability to make specified modifications in the genome to treat some diseases is one of the medical goals as an alternative therapy. Gene therapy is more used to reduce inflammation and improve disease complications in animal models. The ability to transfer genes effectively and induce continuous expression of the transgene protein are the main stages in RA gene therapy (M. Zavvar, et al. 2018). Therefore, gene therapy in RA can be achieved by optimizing selected genes and vectors for accurate and effective delivery of transgenes to target cells. The current gene selection strategies are addressed in the present review.

1.1 Gene delivery strategies
Gene therapy is an intracellular delivery of complementary DNA as a drug and can be mainly administered by two different methods: Local (in vivo) transmission: Involves direct injection of transfer vectors into the joint via intra-articular (IA) injection, and genetic changes occur in the synovial resident cells after harvesting the vectors. Systemic (ex vivo) transmission: In contrast, involves genetic manipulation of specific cells in vitro and reinjection of the modified cells either locally (IA) or systemically (intravenous).

Each approach has advantages and disadvantages (Bandara et al., 1992). In ex vivo methods, different types of cells can be used, hence this is termed as adoptive cellular gene therapy (Bessis et al., 2002). Although in vivo strategies are relatively easy and less expensive, they have a high risk of inducing death, cancer, and infection following necessary gene elimination, oncogene activation, and immune suppression in a nonspecific manner (Woods, Sitabkhan, & Koch, 2008). However, the ex vivo methods have an advantage over the in vivo methods in the ability to target multiple joints simultaneously because of the systemic nature of RA, which typically affects multiple joints.
Table 1. Summary of employed therapeutic genes in RA gene therapy

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Genes</th>
<th>Description</th>
<th>Vector</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro and anti-inflammatory cytokines</td>
<td>sIL-1RACP</td>
<td>Inhibitor of IL-1</td>
<td>Ad</td>
<td>Reduced IL-1 but not TNF levels</td>
<td>Smeets et al., (2003)</td>
</tr>
<tr>
<td></td>
<td>IL-1Ra</td>
<td>Inhibitor of IL-1</td>
<td>Ad</td>
<td>Dose-dependently reduced synovial fluid levels of IL-1a and IL-b</td>
<td>Hur et al., (2006)</td>
</tr>
<tr>
<td></td>
<td>sIL-1RII</td>
<td>IL-1 Type II decoy receptor</td>
<td>Ad</td>
<td>Dose-related inhibition of plasma PGE2 and synovial fluid IL-α and β</td>
<td>Attur et al., (2002)</td>
</tr>
<tr>
<td></td>
<td>sTNFR I, II</td>
<td>Soluble TNF-α receptor</td>
<td>Rv</td>
<td>Suppress cellular immunity and IgG2a anti-collagen antibody</td>
<td>Mukherjee et al., (2003)</td>
</tr>
<tr>
<td></td>
<td>TNF-α shRNA</td>
<td>TNF-α short hairpin RNA</td>
<td>AVV</td>
<td>Suppression of arthritis progression and reduced joint damage</td>
<td>Khoury et al., (2010)</td>
</tr>
<tr>
<td></td>
<td>IL-4</td>
<td>Immunosuppressive cytokine</td>
<td>Ad</td>
<td>Prevented cartilage destruction in CIA</td>
<td>Lubberts et al., (1999)</td>
</tr>
<tr>
<td></td>
<td>IL-18BPc/</td>
<td>Inhibitor of IL-18</td>
<td>Ad</td>
<td>Reduced cartilage/ bone destruction; and IgG2a anti-CII Abs</td>
<td>Smeets, van de Loo, Arntz et al., (2003)</td>
</tr>
<tr>
<td></td>
<td>vIL-10</td>
<td>Inhibitor of TNF-α</td>
<td>Ad</td>
<td>Reduced cell infiltration, proliferation, and pro-inflammatory cytokines</td>
<td>A. M. Woods, Thompson, Wooley, Panayi, &amp; Klavinskis, (2005)</td>
</tr>
<tr>
<td></td>
<td>CXCL10p-IL-10</td>
<td>IL-10 expressing under CXCL10 promoter</td>
<td>LV</td>
<td>Reduced the release of TNF-α and IL-1β</td>
<td>Broeren et al., (2015)</td>
</tr>
<tr>
<td>Component</td>
<td>Role</td>
<td>Adverse Effect</td>
<td>Activity</td>
<td>Reference</td>
<td></td>
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<tr>
<td>IFN-β</td>
<td>Immunosuppressive cytokine</td>
<td>Ad</td>
<td>Reduced arthritis activity and protected against joint destruction</td>
<td>Adriaansen, Fallaux, de Cortie, Vervoordeldonk, &amp; Tak (2007)</td>
<td></td>
</tr>
<tr>
<td>IL-35</td>
<td>Immunosuppressive cytokine</td>
<td>Pl</td>
<td>Induced Treg production with an increased level of IL-10</td>
<td>Thiolat et al., (2014)</td>
<td></td>
</tr>
<tr>
<td>sRAGE</td>
<td>Acts as an anti-inflammatory molecule</td>
<td>Ad</td>
<td>Reduced Th17 cells, increased Treg cells</td>
<td>M. J. Park et al., (2016)</td>
<td></td>
</tr>
<tr>
<td>tsCD35</td>
<td>Truncated soluble complement receptor 1</td>
<td>Rv</td>
<td>Inhibited development of CIA, reduced anti-CII antibody levels</td>
<td>Dreja, Annenkov, &amp; Chernajovsky, (2000)</td>
<td></td>
</tr>
<tr>
<td>Immune deviation and tolerance</td>
<td>Foxp3</td>
<td>Forkhead box p3</td>
<td>Rv</td>
<td>Reduced cell infiltration, pro-inflammatory cytokines, anti-CII Abs</td>
<td>Lan et al., (2012); M. Zavvar (2018)</td>
</tr>
<tr>
<td>IDO</td>
<td>Immunomodulatory activities</td>
<td>Ad</td>
<td>Induced CD4 T-cell apoptosis and reduced synovial IL-17 level</td>
<td>S. Y. Chen et al., (2011)</td>
<td></td>
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<tr>
<td>CTLA-4ig</td>
<td>Binds and blocks both B7 family molecules</td>
<td>Ad</td>
<td>Suppressed established CIA and anti-CII Abs</td>
<td>Quattrocchi et al., (2000)</td>
<td></td>
</tr>
<tr>
<td>ProT</td>
<td>Immunomodulatory activities</td>
<td>Ad</td>
<td>Suppressed chemotaxis and reduced infiltration of macrophage</td>
<td>Shiau et al., (2007)</td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>Immunomodulatory activity</td>
<td>Lv</td>
<td>Induced the generation and/or activation of Treg cells</td>
<td>Delgado et al., (2008)</td>
<td></td>
</tr>
<tr>
<td>TLR 7 shRNA</td>
<td>Toll-like receptor 7 short hairpin RNA</td>
<td>Lv</td>
<td>Reduction in proinflammatory cytokines levels and T-cell infiltration</td>
<td>S. Y. Chen et al., (2012)</td>
<td></td>
</tr>
<tr>
<td>Promoting or inhibiting apoptosis</td>
<td>P53</td>
<td>Regulator of apoptosis and cell cycle</td>
<td>Ad</td>
<td>Induced synovial cells apoptosis and reduced-leukocyte infiltration</td>
<td>Yao et al., (2001)</td>
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<tr>
<td>P16</td>
<td>Involved in regulation of the cell cycle</td>
<td>Ad</td>
<td>Suppression of the expression of pro-inflammatory cytokines</td>
<td>Nasu et al., (2000)</td>
<td></td>
</tr>
<tr>
<td>P21</td>
<td>Regulator of cell cycle progression</td>
<td>Ad</td>
<td>Synovial fibroblasts growth inhibition without inducing apoptosis</td>
<td>Nonomura et al., (2001)</td>
<td></td>
</tr>
<tr>
<td>Trail</td>
<td>Apoptosis inducer</td>
<td>Ad</td>
<td>Reduced arthritis incidence and lower T cells infiltration in the joint</td>
<td>Yao et al., (2003)</td>
<td></td>
</tr>
<tr>
<td>FASL</td>
<td>Interacts with Fas and results in apoptosis</td>
<td>Ad</td>
<td>Induction of synoviocytes and mononuclear cells apoptosis</td>
<td>Kim, Kim, Oligino, &amp; Robbins, (2002)</td>
<td></td>
</tr>
<tr>
<td>FADD</td>
<td>Involved in Fas-mediated apoptosis</td>
<td>Ad</td>
<td>Apoptosis induction especially in the synovium tissue</td>
<td>Kobayashi et al., (2000)</td>
<td></td>
</tr>
<tr>
<td>GAL-1</td>
<td>Induce apoptosis</td>
<td>PI</td>
<td>Reduced anti-CII IgG levels and induced antigen-induced T-cell death</td>
<td>Rabinovich et al., (1999)</td>
<td></td>
</tr>
<tr>
<td>GAL-3 shRNA</td>
<td>Suppress inflammation</td>
<td>LV</td>
<td>Induced antigen-induced T-cell death and decreased T-cell infiltrates</td>
<td>Wang et al., (2010)</td>
<td></td>
</tr>
<tr>
<td>Bcl-x</td>
<td>Antiapoptotic protein</td>
<td>Rv</td>
<td>Induced functional and long-term survival Tregs</td>
<td>Haque et al., (2010)</td>
<td></td>
</tr>
<tr>
<td>SIRT1 shRNA</td>
<td>Contribute to cellular regulation</td>
<td>PI</td>
<td>Silencing of SIRT1 promoted apoptosis in RASFS</td>
<td>Niederer et al., (2011)</td>
<td></td>
</tr>
<tr>
<td>sSTACI&amp;BlyS</td>
<td>A proliferation-inducing ligands</td>
<td>Ad</td>
<td>Reduced the severity of arthritis and anti-CII Abs</td>
<td>Zhang et al., (2002)</td>
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<tr>
<td>TSP1</td>
<td>Inhibitor of angiogenesis</td>
<td>Ad</td>
<td>Reduced clinical severity, synovial hypertrophy, and angiogenesis</td>
<td>Jou et al., (2005)</td>
<td></td>
</tr>
<tr>
<td>TSP2</td>
<td>Modulator of cell-matrix interactions</td>
<td>Rv</td>
<td>Reduced angiogenesis and induced tissue-residing T cells depletion</td>
<td>Y. W. Park et al., (2004)</td>
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<tr>
<td>Endostatin</td>
<td>Fragment of collagen XVIII</td>
<td>LV</td>
<td>Inhibits angiogenesis and pannus formation</td>
<td>G. Yin et al., (2002)</td>
<td></td>
</tr>
<tr>
<td>ATF</td>
<td>Antagonist of upar</td>
<td>Ad</td>
<td>Decreased the incidence of arthritis and the severity of the disease</td>
<td>Apparailly et al., (2002)</td>
<td></td>
</tr>
<tr>
<td>GATA4 shRNA</td>
<td>Regulator of VEGF expression</td>
<td>LV</td>
<td>Diminished synovial inflammation and disease severity</td>
<td>Jia et al., (2018)</td>
<td></td>
</tr>
<tr>
<td>Cyr61 or CCN1</td>
<td>A pro-inflammatory cytokine</td>
<td>Lv</td>
<td>Ameliorate disease and decrease angiogenesis</td>
<td>C. Y. Chen et al., (2017)</td>
<td></td>
</tr>
<tr>
<td>H kallistatin</td>
<td>Angiogenesis inhibitor</td>
<td>Ad</td>
<td>Reduced clinical scores, vessel density, neutrophil infiltration</td>
<td>Wang et al., (2005)</td>
<td></td>
</tr>
<tr>
<td>Angiostatin</td>
<td>Cleaved product of plasminogen</td>
<td>AVV</td>
<td>Reduced hyperplasia, cell infiltration, and angiogenesis</td>
<td>Takahashi et al., (2005)</td>
<td></td>
</tr>
<tr>
<td>sTie2 R</td>
<td>Soluble receptor for Tie2</td>
<td>Ad</td>
<td>Reduced angiogenesis, bone erosion, and levels of RANKL</td>
<td>Y. Chen, Donnelly, Kobayashi, Debusk, &amp; Lin, (2005)</td>
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<tr>
<td>HIF-1α siRNA</td>
<td>Sirna mediated downregulation of HIF-1α</td>
<td>Lv</td>
<td>Reduced in HIF-α and VEGF mRNA levels</td>
<td>del Rey et al., (2009)</td>
<td></td>
</tr>
<tr>
<td>Matrix degradation enzymes</td>
<td>RzMMP1</td>
<td>MMP-1-specific ribozymes</td>
<td>Rv</td>
<td>Decreased the production of MMP-1 and invasiveness of RASFS</td>
<td>Rutkauskaite et al., (2004)</td>
</tr>
<tr>
<td>TIMP1 and 3</td>
<td>Tissue inhibitors of MMPS</td>
<td>Ad</td>
<td>Reduction of the invasiveness of RASF</td>
<td>van der Laan et al., (2003)</td>
<td></td>
</tr>
<tr>
<td>MT1-MMP antisense</td>
<td>Is a crucial promoter of synovial invasion</td>
<td>Rv</td>
<td>Reduced invasiveness of RASFS while inhibition of MT1-MMP protein</td>
<td>Rutkauskaite et al., (2005)</td>
<td></td>
</tr>
<tr>
<td>hTIMP4</td>
<td>Tissue inhibitors of MMPS</td>
<td>DNA</td>
<td>Decreased arthritis development, MMP activity, TNF-α, and IL-1α</td>
<td>Celiker et al., (2002)</td>
<td></td>
</tr>
<tr>
<td>TRIP</td>
<td>Regulator of inflammatory process</td>
<td>Lv</td>
<td>Suppressed NF-κb signaling, proinflammatory cytokines, and MMPS</td>
<td>Kong et al., (2016)</td>
<td></td>
</tr>
<tr>
<td>ODNs</td>
<td>Oligodeoxynucleotides</td>
<td>liposome</td>
<td>Suppressed the production of IL-1 and TNF-α in the synovium</td>
<td>Tomita et al., (1999)</td>
<td></td>
</tr>
<tr>
<td>NBD</td>
<td>Specific NF-κb blocking peptide</td>
<td>pcDNA</td>
<td>Block proinflammatory activation of the IKK complex</td>
<td>May et al., (2000)</td>
<td></td>
</tr>
<tr>
<td>super’ IκB</td>
<td>Inhibitor of NF-κb</td>
<td>Ad</td>
<td>Overexpression enhanced apoptosis in the synovium</td>
<td>Miagkov et al., (1998)</td>
<td></td>
</tr>
<tr>
<td>Molecule</td>
<td>Function</td>
<td>Delivery</td>
<td>Effect Description</td>
<td>Reference</td>
<td></td>
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<td>---------------------</td>
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</tr>
<tr>
<td>IkB kinase-b</td>
<td>Dominant negative form of IKK-b</td>
<td>Ad</td>
<td>Decreased arthritis severity</td>
<td>Tak et al., (2001)</td>
<td></td>
</tr>
<tr>
<td>CIS3/SOCS3</td>
<td>Inhibits JAK tyrosine kinase</td>
<td>Ad</td>
<td>Reduced the severity of arthritis and joint swelling</td>
<td>Shouda et al., (2001)</td>
<td></td>
</tr>
<tr>
<td>JAK/STAT</td>
<td>Overexpression of SOCS3</td>
<td>Ad</td>
<td>Inhibited the arthritic symptoms</td>
<td>Shouda et al., (2001)</td>
<td></td>
</tr>
<tr>
<td>csk</td>
<td>Negatively regulates Src tyrosine kinases</td>
<td>Ad</td>
<td>Is useful in repressing bone destruction and inflammatory reactions</td>
<td>Takayanagi et al., (1999)</td>
<td></td>
</tr>
<tr>
<td>RasDN</td>
<td>Activate MAPK pathways</td>
<td>Ad</td>
<td>Reduced RASFCs proliferation and bone destruction</td>
<td>Yamamoto et al., (2003)</td>
<td></td>
</tr>
<tr>
<td>Cartilage regeneration</td>
<td>FGF-2 Enhancing the repair of cartilage lesions</td>
<td>AAV</td>
<td>Stimulate chondrocyte proliferation over a prolonged period of time</td>
<td>Cucchiarini et al., (2005)</td>
<td></td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Essential in cartilage formation</td>
<td>RV</td>
<td>Stimulate cartilage regeneration</td>
<td>Ha, Noh, Choi, &amp; Lee, (2012)</td>
<td></td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BMP-7</td>
<td>Bone morphogenetic protein-7</td>
<td>Ad</td>
<td>Accelerates the appearance of hyaline-like repair tissue</td>
<td>Hidaka et al., (2003)</td>
<td></td>
</tr>
</tbody>
</table>

Note. The division is based on the title, and it should be noted that some molecules have cross-effects. In addition, codelivery of several genes in combination could result in a synergistic effect in disease amelioration. AAV: adeno-associated virus; Ad: adenovirus; FLS: fibroblast-like synoviocytes; HIF-α: hypoxia-inducible factor α; IL-1: interleukin 1; Lv: lentivirus; MMP: matrix metalloproteinase; mRNA: messenger RNA; RA: rheumatoid arthritis; Rv: retrovirus; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.
1.2 Gene transfer vectors
Gene transfer agents play a very important role in treatment success. Ideal vector choice should be based on two factors: (1) Being efficient in gene transfer, easy entry into target cells, and maintaining stable gene transcription after entry in the nucleus, and (2) being safe, introducing the minimum risk of infection, mutation, and immunogenicity. Recently, many vectors have been developed with one of these properties (Moss, 2014). Therefore, because of the lack of a universal vector, it is necessary to consider the purpose of gene therapy, whether the target is a short or long-term treatment. Generally, strategies for the therapeutic gene transfer can be summarized into two effective methods.

1.3 Nonviral vectors
This collection involves the transfer of a gene through plasmid or synthetic structures of lipids. In addition, to the ability to incorporate large fragments of exogenous DNA, their safety and limited immunogenic potential can be considered as the main advantages (Yin et al., 2014).

1.4 Viral vectors
Generally, four classes of recombinant viral vectors are used as an appropriate means for direct and specific delivery of the therapeutic gene into the cells, including adenoviruses, adeno-associated viruses, retroviruses, and lentiviruses (Giatsidis, Dalla Venezia, & Bassetto, 2013). Many advances have been developed to provide ideal viral vectors by deleting or adding different genes, giving the vector specific features, such as minimal side effects, long-term protein production, easy follow-up, inducible protein expression, and simultaneously multiple protein expression.

2 THERAPEUTIC STRATEGIES FOR RA GENE THERAPY
2.1 Pro- and anti-inflammatory cytokines
The overproduction of proinflammatory cytokines plays a pivotal role in the progression of RA. Therefore, in RA gene therapy great attention is directed to inhibit the intra-articular activity of proinflammatory and overexpression of anti-inflammatory cytokines. In addition to the systemic effects of tumor necrosis factor α (TNF-α), such as increasing the expression of interleukin 1 (IL-1), IL-6, IL-8, chemokines, prostaglandin E2, matrix metalloproteinases (MMPs), and adhesion molecules, it is implicated in joint destruction by activating the stromal cells, chondrocytes, and osteoclasts through RANKL (Kallioliias & Ivashkiv, 2016). Therefore, several attempts have been made to inhibit TNF-α locally in the joint, without causing changes to systemic immune function, through the application of tissue-specific homing T-cell hybridisms (Smith et al., 2003). According to the reported results of the high level of IL-1 in the synovium, the amount of naturally produced IL-1Ra is not sufficient to effectively prevent excessive IL-1 proinflammatory effects (Bessis et al., 2002). It has already become clear that IL-1Ra administration, both locally and systemically, has effects on cartilage and bone turnover and reduces the inflammatory cell infiltration usually seen in arthritis. However, the effects of IL-1 delivery is markedly related to the type of animal models, so it has comprehensive anti-inflammatory and anti-erosive effects in collagen-induced arthritis and adjuvant-induced arthritis, respectively (Robbins, Evans, & Chernajovsky, 2003). Whereas simultaneous inhibition of IL-1 and TNF-α using genetic approaches has been clinically successful, new studies focus on new methods to inhibit structurally and biologically related molecules, such as IL-18, through the enhancement of its natural neutralizer IL-18BPc expression (Smeets et al., 2003). Generally, RA is considered as a Th1 disease, and
therefore, the injection of a viral vector expressing Type 2 cytokine genes could improve RA in various RA animal models. In this regard, according to the anti-inflammatory criteria, interferon β and IL-10 have been considered as appropriate targets for gene therapy as satisfactory results have been reported about them in the treatment of multiple sclerosis and RA (Ma et al., 1998; van Holten et al., 2004). The antiarthritic activities of anti-inflammatory cytokines could be because of their independent anti-inflammatory properties, for example, IL-4, as well as IL-13, are strongly able to protect from bone and cartilage destruction (Lubberts et al., 1999; Woods et al., 2002). Transforming growth factor β (TGF-β), with both immunosuppressive effects and preventing from chondrogenesis, appears to be beneficial for treating RA, but unexpected results (massive fibrosis induction, osteoporosis, and cartilage destruction) were observed (Chernajovsky, Adams, Triantaphyllopoulos, Ledda, & Podhajcer, 1997). Therefore, it seems that TGF-β gene therapy may not be suitable in some RA models (Mi et al., 2003).

2.2 Immune deviation and tolerance
The role of immune cells, such as T and B lymphocytes, as well as the imbalanced ratio of Th1/Th2 and Treg/Th17 cells, is clearly described in RA pathogenesis (Chen et al., 2012). The alternative strategy is the inhibition of the costimulatory molecule interaction by either CTLa-4Ig fusion protein, which reduces T-cell activation (Quattrocchi, Dallman, & Feldmann, 2000), or sCD40-Ig, which induces T-cell apoptosis along with inhibiting B-cell differentiation (Daoussis, Andonopoulos, & Liossis, 2004). This method may not only prevent immediate immune responses but also results in anergy to the epitopes presented in the absence of costimulation. Accordingly, many novel therapeutic approaches are being developed to reduce Th1, Th17, and increase Th2 Treg cell populations (Lubberts et al., 2000). In this regard, there is a considerable focus on costimulatory molecules and chemokines associated with their receptors that are involved in cell function or cytokine production (listed in Table 1). On the other hand, RA pathogenesis is closely correlated with the Th17/Treg cell imbalance (W. Wang et al., 2012). Given the precisely described mechanism, several successful strategies have been proposed, including increasing the Treg cell number by gene transfer of any molecules that can generate Treg cells.

2.3 Promoting and inhibiting apoptosis
It has already been demonstrated that the aberrant apoptosis of fibroblast-like synoviocytes in patients with RA, which in turn facilitates pannus formation, consequently induces destruction of cartilage/bone and protection of infiltrated cells from apoptosis (Korb, Pavenstadt, & Pap, 2009). Some mechanisms are described to be involved in this process, such as increased levels of TNF-α, which activates NF- κB and IL-1β, which in turn induces overexpression of Bcl-2 family protein and mutations of p53 (Rabinovich, 2000). Therefore, genetic synovectomy by targeting various suicide molecules, (listed in Table 1), has been suggested for gene therapy to cover a broader therapeutic spectrum, including synovial cell apoptosis promotion, inflammatory exudates decrease, as well as new matrix deposition (Bokarewa, 2006). Among all transgenic cells, Dendritic cells (DCs) expressing TRAIL has been shown to be more encouraging because of their ability in inducing selective apoptosis of autoreactive T lymphocytes (Liu et al., 2003). On the other hand, there is some evidence for the beneficial role of certain antiapoptotic genes in arthritis. As overexpression of these genes can promote the Treg cells’ life span, their induction might alleviate inflammation in the joint (Gabriel et al., 2016). Induced Tregs exhibit a lower Bcl-2 expression and higher Bim/Bcl-2 ratio, whereas Treg cells
in the synovial fluid of patients with RA express more Bcl-2 and miR-21 compared with conventional Treg cells, which make them more prone to apoptosis (van der Geest et al., 2015; X. Wang et al., 2012). Another gene involved in Treg cell survival and expansion is IL-2R; its signaling is essential for the maintenance of Foxp3 expression (Chinen et al., 2016). According to these findings, modulating apoptotic and cytokine pathways may provide a novel strategy to alter the balance between effector and regulatory T cells in favor of Tregs expansion. The advancements of new therapeutic strategies are aimed at inducing apoptosis in inflammatory cells or blocking apoptosis in engineered cells and seem to be helpful in developing effective clinical interventions.

2.4 Antiangiogenesis
Angiogenesis plays an important role in the pannus formation as an early feature of RA. Angiogenesis is dynamically regulated by the interaction of proangiogenic and antiangiogenic factors. Physiologically, angiogenesis does not occur in an unwanted state because angiogenic inhibitors are dominant. However, this condition of homeostasis is damaged in pathologic conditions. It seems that genetic synovectomy by the expression of endogenous inhibitors, (listed in Table 1) could act as a protective barrier to prevent the penetration of new blood vessels. Therefore, natural angiogenesis inhibitors are attractive candidates for gene therapy in arthritis. Several studies have shown obvious changes in the synovial microvasculature of patients with rheumatoid accompanied by induced-vascular endothelial growth factor (VEGF) levels, which correlates directly with the severity of disease (Elshabrawy et al., 2015; Yi et al., 2016). Expression of VEGF is induced by high levels of hypoxia-inducible factors, which are produced in response to tissue hypoxia (Hua & Dias, 2016). Therefore, inhibiting angiogenesis is likely to be effective in reducing inflammation and disease severity.

2.5 Matrix degradation enzymes
MMPs are a family of zinc-dependent metalloendopeptidase enzymes that are collectively involved in extracellular matrix degradation at neutral pH. Naturally, MMP levels are typically low and are involved in normal physiological processes, whereas induced levels are commonly found in several human autoimmune diseases, including arthritis (Burrage, Mix, & Brinckerhoff, 2006). In the affected joints of RA, synovial fibroblasts are exposed to inflammatory cytokines and produce MMPs, which mediate the degradation of collagen and noncollagen matrix components. It has recently been shown that MMP gene activation in RASF induced by epigenetic changes and cis-elements, contribute to the chromatin state and trans-activation (Araki & Mimura, 2017). Thereby, therapeutic attempts to develop mechanisms that control MMP gene expression, activity, transcription, and messenger RNA (mRNA) stability are under intensive investigation and may provide a new excellent opportunity for the development of therapeutic methods to prevent the common joint destruction seen in arthritis.

2.6 Signaling pathways and transcription factors molecules
Other suitable targets for RA gene therapy can be signaling pathways and transcription factors. As previously reported, some patients with RA have an abnormal lymphoid neogenesis in synovial tissue. As mentioned before, NF-κB is a critical modifier of inflammatory responses in RA (Miagkov et al., 1998). It has been shown that increased transcription of proinflammatory cytokines, adhesion molecules, and inducible nitric oxide genes, as well as the resistance of synovial cells against apoptosis, is followed by high activated NF-κB in the synovium of patients with RA (Tak & Firestein, 2001). Therefore, locally inhibiting NF-κB using different approaches, including injection of the NF-κB decoy,
can significantly reduce RA severity in an experimental RA model. Therefore, it seems that the targeting of NF-κB may suppress hyperplasia in the RA synovium. Accordingly, the usefulness of the gene therapy approach for the inhibition of NF-κB is mixed in other inflammatory diseases (Makarov et al., 1997).

2.7 RNA interference
Gene-silencing molecules, like RNA interference (RNAi), provide a remarkable antisense strategy for highly specific posttranscriptional gene silencing by inhibiting translation or mRNA degradation in various conditions, such as autoimmune diseases. RNAi is a small RNA molecule, which could be classified into three categories based on its origin and function: MicroRNAs, short interfering RNAs (siRNA), and short hairpin RNAs (shRNA). There are basically two strategies to induce RNAi: (1) Introducing presynthesized siRNAs into the target cell, and (2) introducing encoding-shRNA vectors (de Franca et al., 2010). Given the success of recent gene therapy strategies and the possibility of blocking the expression of any protein, there has been a huge interest in the use of RNA-based therapies in a wide range of human diseases, like RA. Accordingly, increasing the stability and specificity of RNAi-based methods, along with reducing undesirable immune responses, depends on a highly accurate and optimal design of these molecules.

2.8 Cartilage regeneration
It has long been known that created immune complexes in RA are deposited in the cartilage and act as the primary source of inflammation. Subsequently, synovitis and pannus are created and eventually lead to cartilage damage as a key feature of RA (Ostrowska, Maslinski, Prochorec-Sobieszek, Nieciecki, & Sudol-Szopinska, 2018). Therefore, intra-articular transferring of growth factors and other protein genes with the capability of cartilage healing may serve as chondroprotective and chondroregenerative medicines to stimulate reparative activities in the joint (Evans et al., 2000). In this category, five groups of genes have been studied: (1) Growth factors, (2) transcription factors, (3) signal transduction molecules, (4) proinflammatory cytokine inhibitors, and (5) apoptosis inhibitors (Tuan, Chen, & Klatt, 2013). This process can be fulfilled by gene transferring to synoviocytes, articular chondrocytes, and mesenchymal stem cells. Strategies that target the synovium may be useful in a chondroprotective form; however, as they do not increase the number of chondrogenic cells within lesions, they may be ill-equipped to repair large defects.

3 DISCUSSION
Multiple immunological proteins, cells, and pathways have contributed to the pathogenesis of autoimmune disease, like as in RA. Therefore, significant advances have been achieved using biological agents as antiarthritic drugs. There is clinically a need for both multiple injections and/or high systemic doses to achieve therapeutic levels in the joint owing to the unique joint physiology, and this process can lead to systemic side effects such as the occurrence of both viral and bacterial infections. However, gene therapy has been used as a novel therapeutic approach not only in arthritis but also in several fields of medicine. Growing interest in gene therapy as an alternative treatment potentially results from the success of the sustained intra-articular delivery of therapeutic products. Gene therapy has been emphasized in RA treatment and, generally, can be functionally divided into inhibition or induction of gene expression. In addition, it can be strategically divided into 12 groups of genes that
are involved in the pathogenesis of arthritis (Figure 1). The promotion of gene therapy as an effective approach in treating RA depends on three important factors: (1) selected treatment genes, (2) vector used for transmission, and (3) routes of administration. Given the clinical manifestations of the disease, the advantages and disadvantages of the two delivery methods (local or systemic) should be considered. The local method is attractive because it has less potential for side effects and treatment is delivered specifically to the inflamed site. However, “created contralateral effects by DC”—a phenomenon in which soluble molecules easily release through the synovial membrane—can play a role in systemic effects (Ghivizzani et al., 1998). In contrast, the rationale for systemic treatment is that RA is a systemic disease, and the theoretical obstacle to use this method is that it requires injection of a higher dose of vectors, which can be accompanied by increasing side effects and toxicity related to the vector and/or to the therapeutic protein (Bessis et al., 2002; Traister & Hirsch, 2008).

![Figure 1](image_url)

**Figure 1** Advantages of selected therapeutic strategies for RA gene therapy schematic illustration of various gene therapy strategies and their main advantages in rheumatoid arthritis. All methods can be strategically divided into 12 groups of genes involved in the pathogenesis of arthritis. At the same time, they can be functionally subdivided into two subspecies including increased (Green) or reduced expression (Blue) of genes. The overall aim of the strategies used is to restore the immune homeostasis in the joint to reduce the inflammation process by infiltrating the immune cells and destroying the cartilage and bone. MMP: matrix metalloproteinase; RA: rheumatoid arthritis

**4 CONCLUSION AND PERSPECTIVES**

Although the results of gene-based studies that have been previously examined may have a positive effect on RA therapy, it is imperative to eliminate many barriers, in order for that gene therapy properly becomes a suitable therapeutic option for arthritis. For example, this method is limited to the early stages or prevention of disease and exerts a minor or no direct effect on the final stages of the disease (Haque, Lei, Xiong, Wu, & Song, 2010). Following this, research efforts should move towards functional studies. Therefore, the therapeutic genes used in subsequent studies likely implicates target genes, which cause inflammation, using a doping gene and delivering specific targeted cells to adapt this technology into a useful clinical treatment modality. On the other hand, subsequent studies need to focus on improving targeted delivery vehicles obtaining the transgene long-term expression and ensuring the safety and efficacy of the currently applied vectors.
Certainly, future advances in biotherapy approaches will reveal the benefits of usage novel gene or pathways such as sex hormones or DNA methylation in the treatment of RA. According to the views expressed in autoimmune diseases such as RA, a number of epigenetic modifications such as altered DNA methylation patterns because of ongoing chronic exposure to inflammatory mediators (Aslani et al., 2016; Mahmoudi, Aslani, Nicknam, Karami, & Jamshidi, 2017) and prevalence association with gender are clearly described (Shahlaee et al., 2015; Sokka et al., 2009). Therefore, it is noteworthy that the development of viral-mediated expression of either sexual hormones or any involved genes in DNA methylation and/or other approaches such as mesenchymal stem cell therapy can serve as a credible and promising immunomodulatory tool to manage and treat RA.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

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