Effects of Smoking on Immunologic and Skeletal Mechanisms Involved in Rheumatoid Arthritis and Responses of Various Biologic Therapies for RA

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Abstract
Smoking is considered a major risk factor in the onset of rheumatoid arthritis. Smokers who also carry the HLA-DR4 shared epitope have a higher risk of development of RA. Smoking releases more than 4000 compounds which not only affect the cardiovascular and respiratory systems but also bone and joint health. Smokers have a higher risk for bone fractures, development of osteoporosis, and degeneration of intervertebral discs. Bone fractures of most smokers heal slower than those of most controls. Smoking also lowers bone mineral density, increases production of proinflammatory cytokines, and augments the risk of citrullination of proteins in the lungs, and possibly in the joints. RA patients who generated antibodies to cyclic citrullinated proteins (CCP) have a higher risk for joint erosions. Although response rates are significantly higher in nonsmoking early RA patients than nonsmoking RA patients with long-standing disease, response rates are not significantly improved in smoking early RA patients. Smoking has decreased response rates to TNF blockers. Additional studies indicated that smoking significantly reduced the response rate of infliximab but not etanercept or adalimumab in RA patients. Because one TNF blocker (infliximab) had significantly lower response rates in a subpopulation of RA patients (smokers versus never smokers) than two distinct TNF blockers, criteria for the development and approval of biosimilars may need to include in vivo trials as well as a demonstration of activity against the primary target (e.g. TNFα). Although the most straightforward recommendation is for patients to stop smoking, investigations on the effect of smoking on response to therapies may serve as a model for elucidating the effect of other environmental contaminants such as air pollution on the response to treatment of flares in RA patients.

Keywords: Smoking; Rheumatoid arthritis; TNFα blockers; Infliximab; Etanercept; Adalimumab; Biosimilars

Introduction
Prognosis for many rheumatoid arthritis (RA) patients has vastly improved in the last two decades due to the introduction of targeted biologic therapies and the improved understanding of the role of lifestyle choices. Long term remission of RA is now a prominent goal for RA management. The ultimate goal is to modulate a critical pathway that subsequently resolves the autoimmune response without globally crippling the immune response of the RA patient to pathogenic agents and transformed cells.

Onset of rheumatoid arthritis is a multistep process [1]. Predisposing factors such as genetic susceptibilities, environmental factors, and immune modulators increase the risk of development of RA [1,2] (Figure 1). Alleles of several genes that modulate the response to external and self antigens (e.g. HLA-DR4 shared epitope, GST1*) increase the risk of RA onset. Low concentrations of vitamin D and essential omega 3 fatty acids may skew an immune response to a microbial threat, and delay resolution of the inflammatory response [3-7]. These predisposing factors can alter gene expression by epigenetic and posttranslational processes [1,8-10]. Multiple causes can initiate the onset of RA in susceptible individuals who are under proinflammatory conditions [2,8]. Previously reported triggering events include infection [11,12], bone fracture [13], exacerbation of food sensitivities [14,15], and overexposure to smoking [16] or other environmental toxins. These events often occur concurrently with a significant emotional event. RA progression is associated with greater alterations in cytokine profiles and regulatory T cells compared to those of healthy controls and patients at RA onset [17].

Because the cause of RA onset or flares in any given patient is unknown to the physician, immunotherapies have traditionally targeted molecules that reduce an aberrant immune response. Current immunotherapies include the TNF blockers (etanercept, infliximab, adalimumab, certolizumabpegol, golimumab), the IL-1 receptor antagonist (anakinra), a blocker of costimulation (abatacept), an IL-6R blocker, and a monoclonal antibody (mAb) that depletes CD20 B cells (rituximab). Efficacy of the biologics ranges from 15%-82% of patients. Because some patients on biologic therapy do not improve and a few patients experience a flare or worse symptoms [18], pursuit of biological markers that correlate with the response to therapy are ongoing. No markers have provided sufficient correlation to warrant their incorporation into clinical decisions. The choice of therapy remains a “trial and error” or trial and learn scenario [19]. Elucidation of a treatment with the highest frequency of efficacy for a given RA subgroup would improve the current “trial and error” decisions for that subgroup. RA patients who currently smoke comprise approx. 20-27% of the British and Swedish RA populations, respectively. Smokers in the US comprised 20.8% of the adult population in 2008, similar to the prevalence of smokers (20.9%) in 2004 [20]. This review summarizes the effect of smoke on the overall RA disease, the immunologic and skeletal mechanisms involved in RA, and the efficacy of various immunotherapies in the subpopulation of RA patients who

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are smokers. This review also notes gaps in the knowledge of the effects of immunotherapies on smokers.

**Smoking’s Effect on RA Disease**

Smoking is a major risk factor for RA onset worldwide and is estimated to be a major factor of RA onset in about 1 in 5 RA patients in Sweden [21] and up to 35% of RA patients who exhibit anti-citrullinated protein antibodies [16,21]. Longer duration and higher intensity of smoking increased the risk of onset of RA [22], especially ACPA positive RA [23]. Smoking was associated with earlier age of onset of RA [24,25]. Smoking also hastened RA progression [26], especially radiographic progression in some studies [27,28] but not others [25,29].

In early RA patients, smoking was associated with higher RA disease activity (number of tender joints, Larsen’s score, DAS-28, higher frequency of autoantibodies), and severity independent of age, alcohol consumption, follow-up duration, and gender [23,24,30]. Prospective monitoring of 100 early RA patients for 2 years indicated that RA disease activity (swollen and tender joints, pain) increased stepwise from never smokers to former smokers to current smokers [31]. Heavy smoking (>20 packs/yr) was significantly associated with greater loss of daily functioning (higher HAQ score, lower grip strength) and development of rheumatoid nodules [28].

Smoking was positively correlated with Rheumatoid Factor (RF) levels, especially IgA RF in one study [28], but not another study [31]. Former or current smokers developed IgA RF more often than nonsmokers [31]. IgARF is associated with higher radiological score for joint erosion [31].

**Smoke**

Cigarette smoke (CS) contains a mixture of approximately 4000 toxic substances including nicotine, polycyclic aromatic hydrocarbons (carcinogens), unsaturated aldehydes, solvents, free radicals, carbon monoxide, and other gases [32]. Nicotine concentration in plasma is <1 mcg/mL in light to heavy smokers while tissues harbor 1-10 mcg/g [33]. Cigarette smoke induces reactive nitrogen species, reactive oxygen species, and reactive sulfur species which can damage proteins, lead to exposure of novel epitopes, and induce antibodies against self-proteins such as type II collagen [34]. In addition, cigarette smoke increases the probability of citrullination of mucosal proteins by peptidyl arginine deiminase type IV (PADI4). PADI4 is overexpressed in synovial fluid of RA patients compared to samples of osteoarthritis [35]. Novel epitopes in the citrullinated proteins (citrullinated fibrinogen, alpha-enolase, vimentin, type II collagen, and filaggrin) can stimulate significant titers of anti-citrullinated protein antibodies (ACPAs). High titers of ACPAs in RA patients correlate with a higher incidence of erosions [36].

**Smoking and Genetic Susceptibility to RA**

In monozygotic twin studies, concordance rate of RA ranges from 12% to 30% [37,38]. Since most twins are raised by their family in the same house with similar diets, lifestyle choices, and environmental exposures, the concordance rate not only includes the contribution of genetic susceptibilities but also subtle differences in the interactions
between their genetics and their predisposing factors, emotional influences [39], smoking [40], and the initiating triggers of RA.

Genetic susceptibility to RA can be grouped into (i) genes that influence the immune and inflammatory responses, (ii) genes that encode enzymes for generation of novel self-antigens [41], and (iii) genes in the detoxification system. Smoking has been shown to increase the risk for developing RA, especially in people who contain the HLA-DRB1 shared epitope in most ethnic groups including Koreans [42], Danes [43], but not all populations such as Brazilians [44]. The shared epitope is found in 31%-70% of RA patients of most ethnic groups, but present in greater than 90% in Native American RA patients [45] and references therein. The shared epitope (SE) is located in the third hypervariable region of HLA-DRB1 (amino acids 70-74) in the following alleles: DRB1*0401, DRB1*0404, DRB1*0405, DRB1*0408, DRB1*0101, DRB1*102, DRB1*1001 and DRB1*1402. Patients who are SE homozygotes and heavy smokers had a 7.47 fold [46] to 50 fold higher risk of developing RA than nonsmoking, SE negative patients [43,47]. Southern European smokers had an earlier onset of RA [24]. Some populations of heavy smokers exhibited a higher risk for anti-citrullinated protein antibody positive RA [47] or for RF positivity and rheumatoid nodules [48] but not ACPA-negative RA. Smokers had a poorer response rate to medication than previous or never smokers in some studies [49] but not all studies [29]. One potential mechanism is that some smokers may induce citrullinated proteins in the joints since smokers induce citrullinated proteins in bronchoaveolar lavage where as nonsmokers did not [47].

ACPA seropositivity is associated with a higher incidence of erosions in RA patients but is not dose dependent [50]. Smokers with RA had significantly higher titers of ACPA than nonsmokers [50]. Analysis of polymorphisms at the PAD4 show that a haplotype containing three polymorphisms in PPDH1 genepad4_89 (rs11203366), pad4_90 (rs11203367), and pad4_92 (rs874881) in Koreans is significantly associated with risk of RA development in both anti-CCP positive RA and anti-CCP negative RA patients [42]. No association with single PAD4 polymorphisms in Caucasians has been reported. Surprisingly, risk of RA in individuals with the PAD4 haplotype or single alleles were not associated with smoking [51].

Glutathione S transferases (GST) and hemeoxygenase 1 (HMOX1) genes encode enzymes involved in inactivation of toxins and their subsequent removal. GST conjugates glutathione to cytotoxic carcinogens and metabolites for efficient excretion. GSTM1 null allele is associated with higher risk for RA [52]. Since smoking produces many toxic substances, Bohane et al. proposed that the null allele of GSTM1 may increase the risk of RA in smokers [53]. The presence of the GSTM1 null allele increased the risk of RA onset in SE homozygotes approx. 8 fold [53]. Smoking increased the probability of Rheumatoid Factor (RF) production in patients with the null allele of GSTM1 [54]. Inducible HMOX1 enzyme, which catalyzes heme into unbound iron, carbon monoxide, and biliverdin, is overexpressed in lesions in synovial tissues from RA patients compared to those of other subjects [55]. It exhibits anti-oxidant, anti-inflammatory and cytoprotective activities. Nicotine reduces HMOX1 levels while increasing proinflammatory cytokines [55]. One polymorphism in the HMOX1 gene promoter (rs2071746) reduces its expression [56].

Smoking and Joints

Smoking has a broad range of negative effects on bone metabolism, including profound bone loss [57]. Smokers have a higher risk for bone fractures [58], development of osteoporosis [57,59], degeneration of intervertebral discs [58,60], and slower healing of bone fractures [58,61]. Bone mineral density values were significantly lower in smokers than those in nonsmokers [62]. Proposed mechanisms include vasoconstriction by nicotine, reduced exchange of nutrients and waste products [60], and direct effects on cells in the joints. Water soluble smoke concentrate (WSSE) reduces cell viability and metabolism of human disc cells, induces an inflammatory response, augments expression of metalloproteinases, and decreases active matrix synthesis and expression of its structural genes in human disc cells [60]. Smokers often have normal levels of markers for bone formation but augmented markers for bone remodeling [57] which is consistent with observations of periodontal bone loss in smokers [62]. Nicotine stimulated metalloproteinase 9 (MMP-9) from a human neutrophil differentiation model, consistent with higher MMP-9 levels found in smokers [63]. It also reduced the size of the oxidative burst from neutrophils and inhibited bacterial killing [63]. Nicotine significantly stimulated TNFα secretion from human osteoblasts [64].

Total protein content, insulin-like growth factor 1 (IGF-1), and beta fibroblast growth factor (bFGF) were significantly reduced in synovial tissue of smokers compared to those of nonsmokers [65]. Smoking significantly reduces the early secretion of tumor growth factor (TGFB) [61] and TNFα levels [66].

Smoking and Immune Responses

Cigarette smoke increases the risk of pulmonary infections, lung cancer, bacterial and fungal infections, as well as the onset of RA. Cigarette smoke modulates both the innate and acquired immune responses. Its effect on cytokines and cell types can differ depending on the species, dose, and conditions of the assay (in vivo, ex vivo, nutritional status, in vitro, smoke components, stimuli and genetic susceptibility). Human lymphocytes exposed to mild (30 mmHg) but not high (200 mmHg) concentrations of smoke showed NF-κB activation, reduction of intracellular glutathione levels, and subsequent enhanced inflammatory gene expression [67,68]. The effects of smoke exposure tracked the nitric oxide (NO) dose-dependent response: low doses activated NF-κB while high NO levels inhibited its activation [68]. Bronchoalveolar lavage (BAL) derived macrophages of smokers showed less clearance of apoptotic cells, reduced glutathione levels, inefficient antigen presentation, and increased proinflammatory cytokines compared to those of healthy controls [69]. In contrast, macrophages from nonsymptomatic smokers showed significantly lower mRNA levels of IL-6, TNFα, interferon gamma, IL-13, and various chemokines (CCL5, CCL3, CCL4, and CCL20) [70]. LPS-stimulated BAL-derived alveolar macrophages from smokers showed reduced secretion of TNFα, IL-6, and IL-8 [71]. However, smoking augments the production of proinflammatory TNFα in the serum [72], but reduces the TNFα production from plasmacytoid dendritic cells in vitro [73].

In addition, smoking significantly affects the transcription of 324 genes in human lymphocytes, including 66 genes involved in cell cytotoxicity (47 negatively correlated and 19 positively correlated), 38 involved in the immune response (30 negatively associated and 8 positively associated), 18 genes for an inflammatory response, 11 in NK cell signaling, 31 for cell activation, 29 for cell adhesion, and 27 for lymphocyte cell proliferation among others [74]. Thus, modulation of additional genes may contribute to smoking’s effect on RA onset and progression.
Smoking may also affect the gut associated lymphoid tissue (GALT) as smokers develop Crohn’s disease at a 1.75 fold higher rate than nonsmokers [75,76]. Interestingly, smoking reduces the rate of ulcerative colitis [75,76]. Since NSAIDs also induce inflammation of the gut and increase intestinal permeability, RA patients who are smokers and utilize NSAIDs for pain relief may be at a higher risk for development of food sensitivities or allergies which can exacerbate RA symptoms in some patients [14].

Smoking and Immunotherapies

Immunotherapies can be grouped into 2 categories: general targeted therapies and antigen-specific immunotherapies. The effect of smoking on responses to therapy in RA has not been systematically examined for most immunotherapies. Although treatment of nonsmokers who were recently diagnosed with RA significantly improved their Disease Activity score in an inverse relationship to duration of RA, smoking obscures or eliminates this effect [49]. Thus, a window of opportunity to obtain higher therapeutic responses in very early RA patients was not evident in smokers [49].

Smoking and General Targeted Immunotherapies

General targeted immunotherapies include inhibitors of cytokines (TNF blockers, IL-1Ra, IL-6 blocker, IL-17 blocker), disruptors of costimulatory signals for T cell activation (Abatacept), a monoclonal antibody (mAb) specific for CD20 that depletes CD20 B cells (Rituximab), and stimulators of regulatory T cells. Overall response rates to biologics for patients resistant to methotrexate is approximately 60-70% and no markers currently predict response to a given biologic although several sets of gene expression profiles are under investigation [77]. Smoking did not significantly increase the rate of switching from a biologic treatment [78]. The most common reasons for switching from a biologic treatment (47%) were inefficacy (43%) and adverse reactions (48%) which included malignancy, rash, infections, or cardiovascular complications) [78].

TNF Blockers

The advent of TNF blockers has revolutionized the treatment of RA. Five currently marketed TNF blockers (etanercept, infliximab, adalimumab, and recently golimumab, certolizumabpegol) reduce TNF signaling by binding TNFα and blocking the binding of TNFα to its cell-bound receptors. TNF blockers utilize three basic mechanisms to remove TNFα from fluids: etanercept is a fusion protein consisting of a soluble TNF receptor 2 linked to a Fc component of human immunoglobulin; infliximab is a mouse-human chimeric monoclonal antibody (mAb) and adalimumab and golimumab are humanm Ab that react with TNFα; certolizumabpegol is a pegylated Fab fragment of a humanized mAb that binds TNFα. Etanercept, infliximab, adalimumab, golimumab provide a moderate or good EULAR response in approximately 50-75% of RA patients in the initial 6 months [79-81]. Certolizumabpegol appears to have a similar efficacy and a higher burden of adverse events [82].

Current smokers with early RA were less likely to respond to treatment with TNF blockers [79,83-85]. Saeverdottir et al. [84] examined the effect of smoking on response to therapy in the pool of early RA patients who had received TNF blocker therapy (infliximab (n=199); etanercept (n=136); adalimumab (n=66)). Current smokers had a significantly lower rate of good response to TNF blockers after 3 months (29%) than never smokers (43%, P=0.05). Previous smokers had an intermediate rate (39%) which was similar to never smokers. Current smoking remained a predictor of higher risk for poor response in multivariate regression analysis at both 3 months (OR: 0.52; CI: 0.29-0.96) and at 6 months (OR: 0.55, CI: 0.31-0.96) in this study population. Because the responses of the patients on TNF blockers were pooled, the efficacy of the three TNF blocking agents in smokers was not compared [84]. The effect of smoking on the response rates of golimumab and certolizumabpegol in RA patients who are smokers remains unclear.

Hyrich et al. examined the factors that correlated with response of RA patients to infliximab (n=1612) or to etanercept (1267) at 6 months [79]. Higher baseline HAQ score, but not age, gender, disease duration, or rheumatoid factor status, was associated with a poorer response to either etanercept or infliximab. In contrast, concurrent use of NSAIDs was associated with a higher EULAR response to these two TNF blockers. In addition, current smokers with RA who were treated with infliximab but not etanercept, were less likely to respond adequately [79]. Multivariate regression analysis indicated that current smoking in infliximab-treated RA patients was a significant inverse predictor of EULAR responses (OR: 0.77, CI: 0.60-0.99); but smoking in etanercept-treated RA patients did not significantly affect responses (OR:1.00, CI 0.77-1.31) [79].

Soderlin et al. [85] compared the 3 month, 6 month, and 12 month efficacy of the three TNF blockers in RA patients who were previous (n=345) or current smokers(n=216) to those who never smoked (n=373). Previous and current smokers had a worse response to TNF blockers as measured by the Simplified Disease Activity Index (SDAI) at 3 months, 6 months, and 12 months than RA patients who were never smokers according to univariate analysis [85]. Whereas the SDAI response to adalimumab was equivalent to the response to etanercept at 3 months, 6 months, and 12 months in this RA patient population according to multivariate analysis, the SDAI response to infliximab was significantly reduced at 3 months (OR=0.56, CI 0.35-0.90), 6 months (OR=0.29, CI 0.15-0.53) and at 12 months (OR=0.36, CI 0.18-0.75) [85]. Heavy smokers showed the least benefit as a group [85].

Two studies [79,85] indicated that the efficacy of infliximab is less in smokers than nonsmokers. Potential mechanisms for the reduced therapeutic benefit of infliximab in smokers include increased citrullination of proteins in smokers, the potential of the citrullinated proteins to induce crossreactive autoantibodies to joint constituents (ACPA), the higher burden of toxins, and higher nutritional needs (e.g. ascorbate, folate) [86-88]. The mechanisms that induce the differential response rate of etanercept and adalimumab in RA patients that are smokers compared to that of infliximab are unclear. The three TNF blockers appear to primarily target soluble TNFα rather than cell associated TNFα. Differences in binding affinities between the three TNF inhibitors do not appear to account for differential response rates in smokers: the binding affinity of etanercept (Kd=0.4 pM) to TNFα was 10-20 fold greater than the affinity of infliximab (4.2 pM) or adalimumab (8.6 pM), yet adalimumab shows similar efficacy in RA patients who are smokers as etanercept [85]. Another possible mechanism is the effect of smoking on apparent clearance of the TNF blockers [89] which can be addressed in future studies.

The effect of smoking on response rate of patients who are ACPA-positive or ACPA-negative and treated with TNF blockers has not been reported. Because one TNF blocker (infliximab) had significantly lower response rates in a subpopulation of RA patients (smokers versus never smokers) than two distinct TNF blockers, criteria for the development and approval of biosimilars may need to include in vivo trials as well as
a demonstration of activity against the primary target (e.g. TNF).

**Effect of Smoking on Response to IL-1Receptor Antagonist (IL-1Ra), IL-6 Blocker, Anti CD-20 Biologic Therapy, and Emerging Therapies**

The effect of smoking on the response to treatment of RA patients with IL-1Receptor Antagonist (IL-1Ra) [90], IL-6 blocker [91], anti CD-20 biologic therapy [92-94], or emerging therapies (JAK inhibitors, cell therapy, tolerogenic dendritic cells, dnaJP1, tolerogenic collagen type II) has not been studied. However, smoke can affect IL-1Ra plasma concentrations [95], IL-1 and IL-1Ra serum concentrations [96-98], IL-6 production [99], and the probability of developing ACPA antibodies (which are a predictor for efficacy with anti-CD20 biologic therapy) [92-94]. In addition, smoking induces heme oxygenase-1 (HO-1) via a JAK2 dependent cascade [100] (which are modulated by kinase inhibitors), does not significantly affect the harvest size of primitive progenitor cells from healthy individuals [101], reduces efficacy of hematopoetic stem cell therapy for cancer [102], and lowers the efficiency of antigen presentation [69]. Thus, studies that directly compare the efficacy of any biologic therapy or emerging therapies in RA patients who are smokers versus the efficacy in nonsmoking RA patients are needed to assist clinicians in choosing medications with the highest probability of efficacy for RA patients who are smokers.

**Conclusions**

Exposure to smoke is a major factor in the onset of rheumatoid arthritis in about 20% of Swedish patients, especially those who carry the HLA-DR4 shared epitope. Smoking releases more than 4000 compounds, some of which affect bone and joint health. Smokers have a higher risk for bone fractures, development of osteoporosis, degeneration of intervertebral discs, and slow healing of bone fractures. Smoking lowers bone mineral density, increases secretion of proinflammatory cytokines, and augments the risk of citrullination of proteins, which increases the risk for joint erosions. Response rates for smokers are similar between early RA patients and those with chronic RA history. Smokers have lower response rates to the TNFα blocker, infliximab but not etanercept or adalimumab than nonsmokers. Criteria for biosimilars may need to include studies that assess responses in smokers as well as demonstrate binding activity to a target protein. The effect of smoking on the response rates to other current immunotherapies or to emerging therapies is currently unknown. Although the most straightforward recommendation is for patients to stop smoking, investigations on the effect of smoking on therapeutic response rates may serve as a model for elucidating the effect of other environmental contaminants such as air pollution, formaldehyde, and volatile organic compounds on the response to treatment of environmental toxin-induced flares in RA patients.

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