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Smoking activates cytotoxic CD8$^+$ T cells and causes survivin release in rheumatoid arthritis

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ABSTRACT

CD8$^+$ T cells have an emerging role in RA. Resent research indicates a causal relationship between the non-exhausted state of CD8$^+$ T cells, defined by lost function of PD-1, and development of arthritis. We investigated how smoking contributes to the non-exhausted phenotype of CD8$^+$ T cells and cause survivin release to serum.

We compared serum survivin levels between smokers and non-smokers in 252 RA and 168 healthy subjects. Nicotine effects on CD8$^+$ T cells were studied in peripheral blood of smoking women, bone marrow of nicotine treated mice and in sorted CD8 spleen cells *in vitro* using flow cytometry and quantitative PCR.

Smoking increased the frequency of survivin release in serum of healthy women (OR 3.64, $p = 0.025$) and in RA patients (OR 1.98, $p = 0.039$). CD8$^+$ T cells of smokers gained a non-exhausted PD-1 deficient phenotype. Expression of the cytotoxic marker CD107 correlated to survivin levels in serum. In the experimental setting, nicotine exposure led to an accumulation of non-exhausted PD-1$^-$IL-7R$^+$ CD8$^+$ T cells in the bone marrow that is abundant with survivin producing cells. The production of the cytolytic protein perforin in bone marrow correlated to serum survivin levels. *In vitro* stimulation of nicotinic receptors on murine CD8$^+$ T cells induced repressive transcription factors T-bet and Blimp-1 in support of the non-exhausted phenotype.

We conclude that nicotine contributes to autoimmunity by supporting the non-exhausted state of CD8$^+$ T cells resulting in the release of survivin. This presents a new mechanism by which smoking may contribute to the pathogenesis of RA.

1. Introduction

The CD8$^+$ T cells are a major lymphocyte subgroup recognized by their cytotoxic effector functions. Activated CD8$^+$ T cells are known to identify and kill cells with MHC class I molecules presenting pathogenic antigens, thereby protecting against infection and cancer. Identification triggers the release of perforin and granzymes that will disrupt the cell membrane and induce apoptosis of the target cell [1]. The cytotoxic activity of CD8$^+$ T cells is controlled by receptors expressed on the cell surface, where the inhibitory receptor Programmed Cell Death 1 (PD-1) plays a leading role [2]. PD-1 activation leads to dephosphorylation of mediators downstream the T cell receptor, thereby inhibiting stimulatory signals following target cell recognition

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[3]. Chronic infection with persistent antigen exposure induces abnormally high expression of PD-1. This impairs the immune response and is referred to as the state of exhaustion [4]. The transcriptional and phenotypic profile of exhausted $CD8^+$ T cells reveal that exhausted cells, in addition to upregulating PD-1, lose the potential to form memory cells, distinguished by expression of the inteleukin-7 receptor (IL-7R) [5]. Exhausted $CD8^+$ T cells loose the capacity to eliminate target cells and inhibition of PD-1 has been approved as a therapeutic strategy to reverse the exhausted state in conditions where a robust immune response is desirable [6].

The $CD8^+$ T cells have been given relatively little attention in autoimmunity, but this seem to be a matter of change [7]. Rheumatoid arthritis (RA) is a canonical autoimmune disease with inflammatory attacks to the joints, often resulting in their permanent damage [8]. In early RA, the number of peripheral blood $CD8^+$ T cell is increased [9] and the $CD8^+$ T cytokine production relate to disease activity [10]. In addition, there is a strong association between seropositive RA and polymorphism in the MHC class I locus [11], supporting a role for $CD8^+$ T cells in autoimmunity. However, reports on $CD8^+$ T cells in RA are inconsistent, and a lower frequency of $CD8^+$ T cells has been described in circulation of arthralgia patients who develop RA and in patients with early RA [12]. Other reports imply no difference in frequency of $CD8^+$ T cells in patients with established RA [10,13]. Exhaustion of $CD8^+$ T cells is reasoned to be a mechanism that sustains peripheral tolerance by controlling the activity of autoreactive cells [14], indicating that induced exhaustion could have a therapeutic potential in autoimmune disease [15]. A consequence of this idea is that the opposite phenomenon, non-exhausted memory-like T cells defined by PD-1 deficiency and high expression of IL-7R, may promote autoimmunity. Indeed, there is a growing amount of evidence supporting a role for PD-1 deficiency in RA. Decreased PD-1 expression has been reported in $CD4^+$ and $CD8^+$ T cells from peripheral blood of RA patients. Low PD-1 expression was associated with higher C-reactive protein levels and disease activity score, indicating a protective role of PD-1 in RA [16]. In fact, it seems like lost PD-1 function contribute to the development of RA. Firstly, polymorphism of the PD-1 gene that affects binding to its enhancer region has been shown in association with RA in Swedish [17] and in Chinese [18] cohorts. Secondly, the use of anti-PD-1 antibodies for treatment of melanoma has resulted in several reported cases of inflammatory arthritis [19–21]. In the experimental setting it was shown that PD-1 deficient mice develop arthritis at a higher incidence and severity [22].

Survivin is an inhibitor of apoptosis protein that can be measured in serum of RA patients to predict aggressive autoimmune disease. Survivin was initially attracting attention due to its overexpression in tumour cells, but several recent studies supports a role for survivin in the immune system [23–25]. Its role in RA was recently discovered when high levels of survivin were found in plasma of both RA patients and pre-symptomatic patients who had not yet developed the disease [26]. In early RA the presence of high serum survivin levels was associated with a poor prognosis and poor response to anti-rheumatic treatment [27,28]. Hence, it is known that the release of survivin is associated with destructive autoimmune processes, but the understanding of what events cause the release is very limited. Survivin can be localised in both nucleus and cytoplasm [29] but is rarely found extracellularly in healthy individuals. The only mechanism for survivin secretion described in the literature is exosomal transport from tumour cells [30]. In this study we hypothesised that extracellular survivin in RA is released when $CD8^+$ cytotoxic T cells target cells with high expression of survivin. This proposal is based on two main arguments: firstly, $CD8^+$ T cells targeting survivin expressing tumour cells is a well known phenomenon in the field of cancer research. The activation of cytotoxic $CD8^+$ T cells by survivin derived peptides aids destruction of survivin presenting tumour cells [31] and has reached clinical trial as a therapeutic strategy for treatment of malignant glioma and metastatic melanoma [32,33]. Secondly, previous results from our lab show that activating survivin specific lymphocytes through vaccination of arthritic mice results in higher serum survivin levels [34], demonstrating the relevance for this mechanism in arthritis.

In the present study we investigate if smoking, and nicotine in particular, shift the phenotype of $CD8^+$ T cells toward a non-exhausted state and promotes the release of survivin.

2. Materials and methods

2.1. Patients

In total, 184 female and 68 male patients of working age with established RA [35] were included in the study between November 2011 and September 2013. Patients were recruited at the Rheumatology units of the Sahlgrenska University Hospital in Gothenburg and the Nothern Älvsborg County Hospital in Uddevalla, Sweden. All but 17 patients were treated with methotrexate (MTX). 81 patients combined MTX with biologics, 18 with other disease modifying drugs. 8 patients used biologics as monotherapy. Oral corticosteroids were used by 23 patients (2.5–15 mg/day). All patients completed a structured questionnaire regarding their smoking habits, medication, and concomitant diseases. At inclusion, all patients underwent clinical examination performed by experienced rheumatologists. Healthy controls consisted of 95 females and 73 males and were recruited from a population study on asthma, the West Sweden Asthma Study (WSAS) [36]. They were examined between 2009 and 2012 using extensive questionnaires regarding their life style, smoking habits, physical examination including lung function tests. Healthy subjects did not report respiratory or autoimmune diseases, or other diseases with systemic inflammation.

Phenotype and transcriptional profile of the peripheral blood $CD8^+$ T cells was analysed in 17 female RA patients (mean age 58.8 (45–76) years, disease duration 14.4 (2–44) years, and in 10 healthy females (mean age 57.9 (49–80) years). At the time of blood sampling, all but 3 patients were treated with MTX (mean 18 (10–25) mg/week) combined with sulfasalazine and hydroxychloroquine in 2 patients; with anti-TNF in 10 patients; with rituximab in 1 patient; and with abatacept in 2 patients.

The study was approved by the Ethical Committee of the Sahlgrenska University Hospital (WSAS, diary no. 593-08; RA, diary no. 659-11). The study was carried out in accordance with the Declaration of Helsinki and patients gave informed written consent prior to participation.

2.2. Mouse models

Two independent experiments were performed. In the first experiment, 20 male, 10 weeks old, DBA1 mice purchased from Taconic Europe A/S (Ry, Denmark) were used. In the second experiment, 38 female, 12 weeks-old Balb/c mice purchased from Charles River (Scanbur, Karlslunde, Denmark) were ovariectomised. Mice were immunised with 100 μg chicken collagen II (Sigma-Aldrich) in complete Freud's adjuvant (Sigma-Aldrich) injected in the tail root. Mice received a booster with incomplete Freud's adjuvant (Sigma-Aldrich) at day 21 in the first experiment. Nicotine (Sigma-Aldrich, St. Louis, MO, USA) was administered in drinking water (0.03%) continuously and control mice drank regular tap