REFERENCE

1. Manivel VA, Mullazehi M, Padyukov L, *et al.* Anticollagen type II antibodies are associated with an acute onset rheumatoid arthritis phenotype and prognosticate lower degree of inflammation during 5 years follow-up. *Annals of the Rheumatic Diseases* 2017;**76**(9):1529–1536. doi:10.1136/annrheumdis-2016-210873

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ABSTRACT WITHDRAWN

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CHARACTERISATION OF THE ANTIBODY RESPONSE TO A CITRULLINATED PEPTIDE DERIVED FROM PORPHYROMONAS GINGIVALIS PAD IN RA

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Introduction Anti-citrullinated protein antibodies (ACPA) – a hallmark of rheumatoid arthritis – antedate joint inflammation. Based on the epidemiological association between RA and periodontal disease (PD),¹ it has been suggested that break of tolerance to citrullinated proteins may occur in the gum mucosa. This hypothesis is primarily based on the unique feature of *Porphyromonas gingivalis* (Pg) – a keystone pathogen in PD – to express a bacterial version of the peptidyl arginine deiminase enzyme (denoted P.PAD), capable of autocitrullination.²

Objectives In the present study, we have investigated the antibody response against CPP3, a citrullinated peptide derived from P.PAD, in order to address the hypothesis that Pg may drive ACPA-production.

Methods

This study included 2,859 RA cases and 4864 controls from the Epidemiological Investigation of RA (EIRA) cohort; 65 PD patients and 59 periodontally healthy individuals; and 218 monoclonal antibodies, derived from RA B cells. Reactivity with citrullinated Pg and human antigens was assayed by ELISA and/or multiplex. Associations with genetic risk factors and smoking were determined by logistic regression. Monoclonal antibody mutations were analysed by IgBLAST comparison. **Results** Anti-CPP3 IgG was detected in 11% of RA, 10% of PD and <2% of controls, with higher levels in RA compared to PD. These antibodies clustered outside the classical ACPA response, associated with smoking, but not with major genetic risk factors. Two CPP3-reactive monoclonal antibodies were identified; one which cross-reacted with citrullinated human vimentin and had extensive mutations, indicating antigen-driven clonal selection and affinity maturation.

Conclusions Based on these data, we propose that Pg infection triggers an antibody response to CPP3, which cross-reacts with citrullinated human proteins by mechanisms of molecular mimicry. Future studies should address whether anti-CPP3 IgG could serve as a biomarker to identify individuals with PD at increased risk for RA.

REFERENCES

1. Fuggle NR, Smith TO, *et al.* Hand to mouth: A systematic review and meta-analysis of the association between rheumatoid arthritis and periodontitis. *Frontiers in Immunology* 2016;**7**:80.
2. Quirke AM, *et al.* Heightened immune response to autocitrullinated porphyromonas gingivalis peptidylarginine deiminase: A potential mechanism for breaching immunologic tolerance in rheumatoid arthritis. *Annals of the Rheumatic Diseases* 2014;**73**(1):263–269.

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P050

HIGH CHOLESTEROL LEVELS BY APOE DEFICIENCY REDUCE BONE DESTRUCTION IN ANTIGEN-INDUCED ARTHRITIS VIA REDUCTION OF THE NUMBER OF OSTEOCLASTS

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Introduction Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by immune complex (IC) deposition in the synovium, leading to increased bone destruction. In RA, joint destruction has been associated with high levels of low density lipoproteins (LDL) and enhanced LDL oxidation (oxLDL). Apolipoprotein E (ApoE) is an important regulator of LDL transportation and its absence strongly elevates circulating LDL levels, which may lead to increased oxLDL levels during inflammation.

Objectives In this study, we investigated the effects of high LDL levels on bone destruction during antigen-induced arthritis (AIA) and how increased LDL/oxLDL levels affect osteoclast formation.

Methods AIA was induced by injection of methylated BSA (mBSA) into the right knee joint of *ApoE*^{-/-} and wild type (WT) control mice previously immunised with mBSA and complete Freund's adjuvant (CFA). WT and *ApoE*^{-/-} Hoxb8 myeloid precursor cells were differentiated into osteoclasts using 20 ng/mL RANKL and 30 ng/mL M-CSF, then stimulated for 24 hour with 10 µg/mL LDL/oxLDL. Oil Red O staining was performed to assess lipid uptake by osteoclasts. mRNA levels of c-Fos, RANK, NFATc1, DC-STAMP, TRAP, CTR, CIC-7 and Cat K were measured by qPCR. Bone erosion was quantified by histological analysis using an arbitrary scale from 0 to 3 and TRAP⁺ cells were determined using immunohistochemistry.

Results *ApoE*^{-/-} mice showed significantly higher LDL serum levels than WT controls. At day 21 after AIA induction, bone erosion was significantly decreased in *ApoE*^{-/-} mice (25% reduction from 1.5±0.2 to 1.1±0.1). In line with that, the number of osteoclasts within the knee joints was 36% lower in *ApoE*^{-/-} mice, as determined by image analysis of TRAP staining. To study the role of ApoE and high LDL levels on osteoclastogenesis in more detail, we differentiated WT and *ApoE*^{-/-} myeloid precursor cells (Hoxb8) into osteoclasts and found similar mRNA levels of osteoclast markers. Whereas LDL stimulation did not affect osteoclast formation, oxLDL strongly impaired cell fusion keeping them in a mononuclear state. mRNA levels of DC-STAMP were significantly down-regulated in both WT and *ApoE*^{-/-} osteoclasts (1.4 and 2.3 fold decrease, respectively) as well as TRAP activity (49% and 58% reduction in WT and *ApoE*^{-/-} osteoclasts), underlining a major role of oxLDL in inhibiting osteoclastogenesis.

Conclusions High LDL/oxLDL levels by *ApoE* deficiency affect bone destruction during AIA by reducing the number of osteoclasts in the synovium, probably by interfering with osteoclastogenesis.

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LOSS OF BONE MASS IN PATIENTS WITH HEAD AND NECK TUMOURS IN THE FIRST YEAR AFTER THE DIAGNOSIS OF THE DISEASE

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Introduction Head and neck tumours (TTCC) constitute a heterogeneous group of diseases that affect the otorhinolaryngological sphere. The risk factors such as tobacco and alcohol are also for osteoporosis too. The cancer itself, with the malnutrition suffered after surgical treatment and chemoradiotherapy, represents a potential situation of bone loss with the subsequent appearance of osteoporosis and its complication 'the fracture'. We do not have data on the frequency of osteoporosis in patients with head and neck tumours.

Objectives To evaluate the loss of bone mineral density (BMD) in the lumbar spine during the first year of follow-up after diagnosis in a cohort of patients with head and neck tumours.

Methods Observational, longitudinal and prospective study in a cohort of patients with TTCC at the Virgen de Valme hospital, one year follow-up. Bone mineral density was measured by DXA in the lumbar spine before the start of treatment and once a year. To identify qualitative factors that determine bone loss (<2%/≥2%), contingency tables are performed and the Chi-Square test or the non-asymptotic methods of the Monte Carlo test and the Exact test are applied.

Results A total of 50 patients were included. 100% of the patients were male. The average age at diagnosis of 60.9±7.8 years. 98% were smokers, and 76% habitual heavy drinkers. 12% had a family history of hip fracture. 18% have a disease that causes malabsorption syndrome.

The median BMD at baseline is 1.05 g/cm², and after one year of follow-up this median decrease is 1.02. The loss of bone mass in a year expressed in median is estimated at 0.03 g/cm², which corresponds to a loss of bone mass per year of 2.8%, which reflects a greater loss than that of a population of same age and sex estimated at 0.5–1% per year.

Although the loss suffered by our patients is not from a significant statistical point of view, given the sample size, it does reflect a tendency to increase the risk of osteoporosis in these patients.

Conclusions Patients with TTCC suffer a bone loss greater than expected in a population of equal age and sex, in the first year of follow-up. Initial screening of these patients would be indicated, which would include OP and/or fracture risk factors.

REFERENCE

1. Valero C, Olmos JM, Rivera F, Hernandez JL, Vega ME, Gonzalez Macias J. Osteoprotegerin and bone mass in squamous cell head and neck cancer patients. *Calcif Tissue Int* 2006;78:343–347.

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P052

EFFECTS OF HYPOXIA AND ACTIVATED HUMAN B CELLS ON IMMUNOSUPPRESSIVE AND DIFFERENTIATION CAPACITY OF MULTIPOTENT MESENCHYMAL STROMAL CELLS

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Introduction Multipotent mesenchymal stromal cells (MSCs) are a promising approach for the treatment of chronic inflammatory autoimmune diseases. However, little is known about whether their immunomodulatory, replicative and regenerative potential is influenced by the pathogenic environment.

Objectives Activated lymphocytes and hypoxia are part of the cellular environment in chronic inflammatory diseases. The aim of the study was to investigate the influence of activated B cells and hypoxia on MSCs functions.

Methods Bone marrow MSCs from healthy donors were cultured for various time periods under normoxia or hypoxia (3% O₂) in presence or absence of B cells from healthy donors activated via B cell receptor cross-linking and IL-2. Alternatively, cell culture supernatant from B cells activated for three days with respective stimuli was used. Cell division and expression of surface markers were assessed by flow cytometry, cytokine secretion by ELISA and gene expression by qRT-PCR. For differentiation, MSCs were cultured for 21 days in established differentiation media in presence or absence of B cell culture supernatant. Osteogenesis and adipogenesis were assessed by fluorescence microscopy and quantified with ImageJ software.

Results Significant increase of MSC IL-6 and IL-8 production over more than 100-fold in coculture with B cells revealed activation of MSCs by B cells both under normoxic and hypoxic conditions. In contrast, B cell specific TNF-α production was reduced by more than half, demonstrating suppression of B cell activation by activated MSCs in return. This was observed both in cocultures with direct contact and indirect contact upon separation by cell culture inserts, suggesting soluble factors were sufficient for observed effects. Both hypoxic conditions and coculture with MSCs significantly inhibited B cell division, without increasing apoptosis. B cell CD25 expression significantly decreased in cocultures, but remained higher under hypoxia. B cell supernatant increased MSC proliferation rate and delayed entry into senescence and this effect was more pronounced under hypoxia. Of note, B cell-derived factors increased MSC-specific expression of hypoxia induced transcription factor HIF-1α under normoxic conditions. B cell derived factors also supported osteogenic differentiation and inhibited adipogenesis both under normoxia and hypoxia.

Conclusions These results suggest an important influence of the inflammatory environment on MSCs functions: In presence of B cells MSCs exert immunosuppressive properties, but also secrete high amounts of proinflammatory cytokines. In addition, the replicative potential of MSCs increases, which is further supported by hypoxia. In presence of activated B cells, MSCs differentiation is governed towards the osteogenic lineage and this effect is stable both under normoxia and hypoxia.

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