Joint Bone Spine 83 (2016) 43–46

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com/en

Original article

Presence of HLA-B27 is associated with changes of serum levels of mediators of the Wnt and hedgehog pathway

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ARTICLE INFO

Article history:
Accepted 25 March 2015
Available online 19 October 2015

Keywords:
HLA-B27
Bone
Spondyloarthritis

ABSTRACT

Background: HLA-B27 is present in 5% of the Caucasian population and is strongly associated with the development of spondyloarthritis (SpA), a disease characterized by inflammation and substantial bone changes. We hypothesized that the presence of HLA-B27 in itself is associated with alterations of key regulatory of bone homeostasis.
Methods: Sera of 241 individuals were assessed for the serum levels of Wnt pathway regulators, sclerostin and dickkopf (Dkk)-1 as well as Indian hedgehog (IHH) and collagen type I cleavage products (CTX1). Of the 151 HLA-B27+ subjects, 31 had SpA, 30 had anterior uveitis, 30 were healthy individuals and 60 healthy siblings of patients with SpA.
Results: Sclerostin levels were significantly ($P < 0.001$) lower in HLA-B27+ subjects (314 ± 21 pg/mL) compared to HLA-B27 negative controls (mean ± SEM: 492 ± 30 pg/mL), no matter if subjects were either healthy, or affected by SpA or uveitis. Similar results were found for Dkk-1. No differences between the groups with respect to the bone resorption marker CTX1 were found. In contrast, IHH levels were significantly ($P < 0.001$) higher in the carriers of HLA-B27 than in the negative controls.
Conclusions: Changes in key regulators of the Wnt pathway as well as IHH, a molecule regulating endochondral ossification, are found in HLA-B27 carriers, independent if they were healthy or affected by uveitis or SpA.

1. Introduction

About 5% of the Caucasian population share the human leucocyte antigen B 27 (HLA-B27), a specific version of the major histocompatibility (MHC) class I complex [1]. Subjects bearing HLA-B27 are at risk to develop spondyloarthritis (SpA) and diseases associated with SpA, such as anterior uveitis. Long before modern genetics, HLA-B27 was known as the key genetic factor associated with SpA and the later performed large GWAS studies confirmed MHC class I alleles as by far the strongest genetic factor linked to SpA [2,3]. These findings not only allowed using HLA-B27 in clinical practice, as it has become an important factor for the classification of SpA, but have also suggested that HLA-B27 represents a true clue to the pathogenesis of the disease apart from a mere association. In support of this concept, rats transgenic for HLA-B27 spontaneously develop SpA-like inflammation and osteoproliferation [4].

The mechanisms by which HLA-B27 facilitates the development of SpA and associated diseases are not fully understood [5]. Its expression on antigen-presenting cells however suggests that these cells may show an altered function when expressing HLA-B27. Several theories were set up in the last decades amongst them, which altered peptide presentation by HLA-B27-bearing cells to CD8 T cells as well as biological changes arising from an abnormal folding of the HLA-B27 heavy chains were the most appealing concepts. Particularly, enhanced degradation of misfolded HLA-B27 heavy chains in the endoplasmatic reticulum (ER) has been discussed as a mechanism, as it triggers an unfolded protein response in the cells linked to deregulated cytokine expression.

SpA is characterized by enthesial inflammation and bony proliferation. In the last years, substantial progress has been made

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http://dx.doi.org/10.1016/j.jbspin.2015.03.019
1297-319X/

to understand the pathophysiology of both processes, by linking enthesial inflammation with the expression of IL-23 and explaining bony proliferation by activation of Wnt, BMP and hedgehog proteins [6–10]. Apart from experimental studies also clinical data have supported these notions showing that:

- IL-23 and downstream IL-17 are inducibly expressed in the tissue of SpA patients [11];
- clinical responses to IL-23 and IL-17 inhibition in patients with SpA [12];
- the predictive value of molecules regulating bony proliferation in the development of ankylosis in patients with SpA [13,14].

The strong association between HLA-B27 and SpA at one side and the pronounced and specific bone changes in SpA on the other side raises the question whether the presence of HLA-B27 may affect key players of bone metabolism. The observation that within the SpA patient group, those positive for HLA-B27 tend to have a more severe disease course with more signs of osteoproliferation supporting such concept. It is however difficult to judge whether HLA-B27 plays a role in bone homeostasis when focusing on SpA only, since inflammatory changes in the bone, which is a typical feature of SpA, and clinically apparent osteoproliferation may affect the bone markers. Hence, studying healthy subjects positive for HLA-B27 and also HLA-B27-positive patients with anterior uveitis may help to address this question in a more stringent way.

To investigate whether HLA-B27 affects key players for regulation of osteoproliferation in SpA, we therefore not only focused our analyses on SpA patients, but also included HLA-B27-positive healthy individuals and anterior uveitis patients in our analysis comparing them to HLA-B27-negative healthy individuals. We assessed the serum levels of two key Wnt antagonists, sclerostin and dickopf (Dkk)-1, previously shown to play a role in bone changes in SpA and additionally analyzed the bone resorption marker CTX1 and Indian hedgehog (IHH), a protein involved in hypertrophic chondrocyte formation.

2. Methods

2.1. Patients characteristics

Sera of 241 subjects were investigated in this study, 90 of them were HLA-B27 negative controls, 151 were carriers of HLA-B27+. Of them, 31 were classified as SpA according to the ASAS criteria [15] and 30 had isolated anterior uveitis without any joint complaints. The additional 90 HLA-B27+ subjects were healthy individuals, 30 were recruited based on HLA typing in Erlangen, 60 were identified as healthy HLA-B27+ siblings of patients with SpA in Paris. Demographic characteristics (age, sex) were assessed in all subjects. Furthermore, smoking habits and presence of diabetes were recorded in uveitis, SpA and healthy HLA-B27 positive subgroups. In the SpA group, disease duration, visual analogue scale for patient global disease activity and presence of peripheral disease with assessment of tender and swollen joint counts were determined. Approval from our local ethics committee at the University of Erlangen-Nuremberg as well as written informed consent of the participants was obtained for the study. The study was performed in accordance with the Declaration of Helsinki.

2.2. Serum analysis

C-terminal collagen type I cleavage products (CTX1) were assessed by an enzyme-linked immunosorbent assay (ELISA) from Nordic Bioscience (Herlev, Denmark). The intra- and inter-assay precisions (CV%) were <7% and <12%, respectively. Sclerostin and Dkk-1 serum levels were analyzed by ELISA from Biomedica (Vienna, Austria). The intra- and inter-assay precisions for sclerostin were <7% and <10%, those for Dkk-1 were <3% and <3%, respectively. IHH level was measured by an ELISA from Cusabio (Wuhan, China). Intra-assay precision was <8%, inter-assay precision was <10%. All parameters were assessed in all 241 subjects, with the exception that HLA-B27-positive healthy siblings from SpA patients were only assessed for sclerostin and IHH levels.

2.3. Statistics

After descriptive data analysis, we performed inferential two sided *t*-tests for independent samples to investigate between group differences in the four assessed parameters. In order to determine whether regular or Welch-corrected *t*-tests will have to be carried out, we previously calculated Levene's *F*-Tests to evaluate the required homogeneity of variances. The problem of multiple tests was accounted for by Bonferroni–Holm correction. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Demographic characteristics

Age and sex distribution was similar among the five groups of subjects analyzed in this study, which included:

- HLA-B27 negative healthy controls (mean ± SD age: 50.9 ± 15.6 years);
- HLA-B27 positive SpA patients (mean ± SD age: 47.8 ± 16.3 years);
- HLA-B27 positive anterior uveitis patients (mean ± SD age: 46.0 ± 12.9 years);
- HLA-B27 positive healthy individuals (mean ± SD age: 51.9 ± 12.6 years);
- HLA-B27 positive healthy siblings of SpA patients (mean ± SD age: 47.1 ± 10.7).

Prevalence of smoking (15.6%) and diabetes (3.3%) did not differ among the groups. Mean disease duration was 6.3 ± 10.2 years in the SpA subgroup. Visual analogue scale for SpA patients global disease activity was 18 ± 24.1. Fourteen (45.2%) of the SpA patients had concomitant peripheral disease with a mean ± SD tender joint count of 5.1 ± 6.1 and a swollen joint count of 1.6 ± 2.0.

3.2. Bone resorption marker

We first analyzed CTX1, a marker of bone resorption in the various groups of subjects. Mean (± SEM) serum level of CTX1 in HLA-B27 negative controls was 573 ± 18 pg/mL. HLA-B27 positive groups, including healthy individuals (524 ± 21 pg/mL, $P = 0.151$), anterior uveitis patients (629 ± 37 pg/mL, $P = 0.156$) and SpA patients (583 ± 19 pg/mL, $P = 0.782$) did not differ in their CTX1 level from the controls. Moreover, when pooling all HLA-B27+ groups, serum level of CTX1 (578 ± 16 pg/mL) was not different between HLA-B27 carriers and HLA-B27 negative controls (Fig. 1).

3.3. Serum level of Wnt antagonists

We next compared the serum levels of the Wnt antagonist sclerostin, an inhibitor of bone formation in the five groups of patients. Sclerostin level was highest in HLA-B27 negative controls (mean ± SEM 492 ± 30 pg/mL). All HLA-B27 positive groups, including healthy individuals (289 ± 45 pg/mL, $P < 0.001$), healthy siblings (377 ± 40 pg/mL, $P = 0.023$), anterior uveitis patients (306 ± 42 pg/mL, $P < 0.001$) and SpA patients (223 ± 32 pg/mL,