Identification of Circulating Natural Antibodies against Endogenous Mediators in the Peripheral Blood Sera of Patients with Osteoarthritis of the Knee: A New Diagnostic Frontier

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Abstract

Introduction: The presence of NA against EM regarding specificity and have gained increasing attention in proteome analysis for diagnosis, developing, monitoring and effective treatment of osteoarthritides of the knee (kOA). An understanding of the various regulatory systems controlling blood vessel growth, inflammation and pain in the joint should lead to help explain kOA disease progression. To investigate the specific presence of NA (IgM, IgG, IgA) against EM (BK, All, VEGF, bFGF) in the sera of kOA patients and control and to correlate this with process of joint destruction.

Methods: In this study novel immunoconjugates were designed, synthesized and then used to develop a rapid, specific and sensitive ELISA method to directly detect immune complexes (NA-EM) in humans. Following this procedure, we examined variations in the levels of natural antibodies recognized a panel of self-antigens in the sera from healthy individuals and kOA patients. Blood samples were obtained from 250 patients with symptomatic kOA and 250 ages, sex-matched healthy individuals.

Results: NA against EM was detected with novel ELISA assay in the sera of kOA patients as well as in the sera of control. At time of inclusion kOA patients (100%) had significantly higher BK-IgG levels relative to normal sera. The over expression BK-IgG were positively associated with destructive changes (KL>4; r=0.75; p<0.005). kOA patients in whom KL scores progress rapidly tend to have higher BK-IgG levels at all time point. Serum BK-IgG over expression in kOA patients were positively associated with destructive changes (KL>4; r=0.75; p<0.005). Elevated BK-IgG was significantly correlated with VAS (r=0.85; p<0.0001) and loss of functions (r=0.69; p<0.0003) in kOA patients. Affinity chromatography yielded EM-specific NA from the sera of healthy individuals and kOA patients.

Conclusions: We showed that EM represents a group of novel self-antigens which are targeted by NA from kOA patients. Circulating BK-IgG in the sera has been proposed as a sensitive and specific marker of diagnosing kOA at early stages of the disease. Our results have potential applications for controlling unwanted angiogenesis, inflammation, pain and future response to therapy in kOA patients.

Keywords: Osteoarthritis of the knee; Angiogenesis; Vasoregulatory systems; Inflammation; Pain; Radiological damage; immune regulation; Natural antibodies; Endogenous mediators; Serodiagnosis; Biomarkers

Abbreviations: ABTS: 2,20-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid); AII: Angiotensin II; BFGF: Basic Fibroblast Growth Factor; BK: Bradykinin; BK-IgG: Natural IgG Antibodies against Bradykinin; BSA: Bovine Serum Albumin; CT: Computed Tomography; ELISA: Enzyme Linked Immunosorbentassay; EM: Endogenous Mediators; EM-IgG: Natural IgG Antibodies against Endogenous Mediators; HMW: High Molecular Weight; HAS: Human Serum Albumin; Ig: Immunoglobulin; IgA-HRP: Immunoglobulin A-Horseradish Peroxidase Conjugate; IgG: Immunoglobulin G; IgG-HRP: Immunoglobulin G Horseradish Peroxidase Conjugate; IgM: Immunoglobulin M; IgM-HRP: Immunoglobulin M-Horseradish Peroxidase Conjugate; KL Grade: Kellgren-Lawrence grade; KKS: Kallikrein-kinin System; kOA: Osteo Arthritis of the knee; LMW: Low Molecular Weight; NA: Natural Antibodies; NHS: Normal Human Sera; OA: Osteoarthritides; PBS: Phosphate-Buffered Saline; PBST: phosphate-buffered saline with Tween20; RA: Rheumatoid Arthritis; RAS: Rennin-Angiotensin System; TMB: 3,3’,5,5’-TetramethylBenzidine; VAS: visual analogue scale; VEGF: Vascular Endothelial Growth Factor

Introduction

Osteoarthritis (OA) is the most common disorder affecting synovial joints, with structural changes of osteoarthritis present in approximately half of the adult population in world [1]. The knee is the most commonly affected weight-bearing joint and various deformity is the most common misalignment of the knee associated with osteoarthritis.

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Osteoarthritis of the knee (kOA) can be a progressive disabling disease, which results from the pathological imbalance of degradative and reparative processes, with concomitant inflammatory changes [2]. The clinical features of kOA include pain, stiffness, reduced motion, swelling, crepitus, and deformity [3-5].

Several factors are considered for the pathogenesis of kOA [6-14]. Complicated path biological interactions between the Kallikrein-Kinin System (KKS), the Rennin-Angiotensin (RAS), angiogenesis and natural immunity could contribute to joint destruction in the disease process of kOA [15-19]. It is also likely that biomarkers will be used in conjunction with imaging in order to establish stage of disease, predict progression, and assess improvement in the setting of clinical trials [20-22].

Vasoregulatory systems are developed and a fully functional microvasculature is formed in kOA [6,19,23,24]. Vasoregulatory systems, as the KKS and the RAS, play essential roles in the maintenance of vascular homeostasis [25,26]. An understanding of the various regulatory systems controlling blood vessel growth, inflammation and pain in the joint should lead to help explain kOA progression.

The KKS, most well-known as mediator of inflammation, also has role in the control of vessel diameter and growth [27,28]. Factors of KKS are thought to be key mediators in inflammatory joint disease [3]. Kinins released into synovial fluid by the proteolytic action of kallikreins on kinogenins on the surface of neutrophils are likely to cause vasodilatation and pain, increase vascular permeability, promote leukocyte margination and stimulate cytokine release from monocytes [17,29]. Pain, the predominant symptom in kOA, is multidimensional in its nature and mediated through a variety of factors as bradykinin [30-33].

The RAS is best known as a major regulator of blood pressure, but it also is important at the micro vascular level in the regulation of neovascularization [34,35]. In addition, the RAS has important modulatory activities in the process of kOA [24]. Vascular inflammation is an independent risk factor for the development of kOA [19]. Angiotensin II (All) augments vascular inflammation, induces endothelial dysfunction, and, in so doing, enhances the process of kOA [25]. All the classical signs of inflammation-pain, redness, erythema, edema, and hyperthermia.

Angiogenesis, defined as the development of new capillaries from preexisting blood vessels, is an important the pathogenesis of kOA since it in the initiation and perpetuation of the disease [2,18]. Inflammation and angiogenesis are closely associated in kOA, modulating functions of chondrocytes, contributing towards abnormal tissue growth and perfusion, ossification and endochondral bone towards abnormal tissue growth and perfusion, ossification and endochondral bone development, leading to radiographic changes observed in the joint [36-39].

Almost all of the human autoimmune diseases are characterized by the generation of Natural Antibodies (NA) [40-44]. Identifying those antibodies is the cornerstone for the diagnosis of autoimmunity in humans [45-50]. In autoimmune rheumatic diseases, pathogenic auto antibodies are used for classification, development of diagnostic criteria, monitoring of disease activity and prediction of prognosis [51-53]. However, autoimmunity defined by the detection of auto antibodies does not necessarily imply the presence of an autoimmune disease. Furthermore, the normal immune system is able to produce, in relatively high amounts, antibodies that bind various self-antigens, i.e. endogenous mediators. Those auto antibodies, defined as NA, have an important physiological regulatory role [54-57].

NA refers to antibodies that are present in the serum of healthy individuals in the absence of deliberate immunization with the target antigen [58,59]. A vast majority of NA react with one or more self antigens and are termed as natural auto antibodies [60,61]. The importance of NA in immune regulation has long been neglected, since tolerance to self was thought to be primarily dependent on the deletion of auto reactive clones, rather than on peripheral suppressive mechanisms [62,63]. Clonal deletion and energy cannot account, however, for the prevalence of natural auto reactivity among healthy individuals [64]. It is now well established that auto reactive antibodies and B cells, and auto reactive T cells, are present in healthy individuals, and in virtually all vertebrate species [65]. Auto reactive repertoires are predominantly selected early in ontogeny [66,67]. Questions pertaining to the role of NA in the regulation of the immune response and maintenance of immune homeostasis and to the distinction between natural auto reactivity and pathological autoimmunity have not been adequately addressed [68,69].

KOA is a chronic, destructive autoimmune disease of the joints [9,70,71]. It is characterized by the presence of NA that are reactive to various target molecules [72-76]. kOA is an autoimmune disease characterized by chronic synovitis, which manifests as joint pain and often progresses to bone and joint destruction [77-81]. Inflammation in kOA may result from a number of different mechanisms, including antibody-mediated complement activation and cellular injury, T-cell-mediated mechanisms and generation of pro-inflammatory mediators [82,83].

Differentiating between pathogenic, natural and other nonpathogenic auto antibodies is crucial for the definition, diagnosis and identification of a reliable biomarker of osteoarthritis [84-87]. The identification of NA that highly predicts the development of kOA is of great interest. This study focuses on human NA against EM discovered in our Unit [88,89]. Development technologies that permit assessment of potentially disease-modifying agents of vascularization and inflammation are the current approach to the management of kOA. It is an established fact that any physiological stress can interact with the immune system.

The analysis of the osteoarthritis-associated antigen-antibody systems in the normal peripheral blood has new approach to the patient at risk for or with newly diagnosed kOA. The presence of NA against EM regarding specificity and have gained increasing attention in proteome analysis for diagnosis, developing, monitoring and effective treatment of kOA. Previous retrospective studies in different countries have shown that NA can be detected in patients with kOA several years before clinical symptoms occur [57,90-93]. Given the low prevalence of kOA, NA testing in the general population is of no clinical benefit. NA in kOA has been found to be quite useful in clinical practice for diagnosis and assessing prognosis. NA against EM has recently been shown to predict development of kOA as well as poor outcome in early kOA.

The aim of this study was to investigate the specific presence of NA (IgM, IgG, and IgA) against EM (bradykinin, angiotensin II, vascular endothelial growth factor, basic fibroblast growth factor) in the peripheral blood sera of patients with kOA and healthy individuals. In addition, we are interested in investigating the functional properties of affinity-isolated NA against EM from the peripheral blood of healthy individuals and patients with kOA (focusing on isotype, affinity,
specificity). This study also further characterizes the markers of clinical relevance in patients with kOA.

Methods

Patients

The study included 250 patients with symptomatic kOA (age range 45-79 years) fulfilling the American College of Rheumatology criteria for kOA. All patients with kOA had involvement of the knee joint with typical radiographic changes graded Kellgren Lawrence classification. Pain was scored on a Visual Analogue Scale (VAS) immediately after walking 50 m. All patients with kOA are with persistent pain longer than 6 months. Parameters for function were performed by Lequesne's functional indexes. 250 age and sex-matched healthy Individuals. Characteristics of patients are listed in (Table 1). The patients and control had no associated organic disease and exhibited no evidence of autoimmune disease. They did not have immunological or other arthritic disease or any physical illness known to affect their immunological status.

Control subjects

The general reference (normal) control samples consisted in age- and sex- matched 250 healthy individuals in Blood Transfusion Service of the National Institute of Rehabilitation (Mexico City, Mexico). The absence of disease was confirmed by physical examination, clinical history and routine laboratory tests.

Blood sampling

Seven milliliters of the peripheral blood was drawn into a serum separator tube (Vacutainer Systems, code 607213 Becton-Dickinson, USA). Blood was allowed to clot for 1 h at Room Temperature (RT). Sera were obtained after centrifugation at 3000 rpm for 10 min at 4°C. All serum samples were stored in 30 µl aliquots at -80°C until analysis.

Reagents

All reagents were of analytical grade and were obtained from Sigma-Aldrich Ltd, Poole, UK, unless otherwise indicated.

Design and synthesis of immunoconjugates for ELISA

In this study novel immunoconjugates were designed, synthesized and then used to develop a rapid, specific and sensitive ELISA method to detect NA against EM directly in the peripheral venous blood sera of humans. Human low molecular weight EM was coupled with High Molecular Weight Matrix (HMWM: polyphenylacrilate) according to in-hous protocols provided by Tissue Engineering, Cell Therapy and Regenerative Medicine Unit (National Institute of Rehabilitation, Mexico City, Mexico). The conjugated EM-HMWMM was then dialyzed against Phosphate Buffered Saline (PBS), pH 7.4 at 4°C.

Development of ELISA for rapid detection natural antibodies against endogenous mediators

Poly styrene micro titer ELISA plates with 96-wells (Maxi-sorb, NUNC, Rochester, NY, USA) were incubated overnight at 4°C with EM-HMWMM (1 µg/ml) in 0.1 M carbonate/bicarbonate buffer, pH 9.6. The final volume of this as well as of all other steps was 100 µl per well, unless stated otherwise. After washing the plates twice with PBS, residual binding sites were blocked (1 h at RT) with 200 µl per well of PBS containing 2%, w/v, Human Serum Albumin (HAS). Human sera were appropriately diluted in assay buffer (veronal buffer containing 0.1%, w/v, HSA, 2 mM CaCl2, 0.1%, w/v, Tween-20, pH 7.4), and, incubated for 1 h at RT. After this and the subsequent incubation steps, the plates were washed with PBS containing 0.1%, w/v, Tween-20 (PBST). IgM, IgG, IgA bound to EM-HWMW was quantified with horseradish peroxidase labeled anti-human IgM, IgG, IgA diluted in assay buffer. Finally, horseradish peroxidase activity was visualized by incubation with 100 µg/ml 3,3’5,5’-Tetra-Methylbenzidin (TMB), in 0.11 M sodium acetate, pH 5.5, containing 0.003%, v/v, H2O2. The reaction was stopped after 10 min by addition of 2 M H2SO4 and the absorbance at 450 nm was measured in a micro titer plate reader (Bio-Kinetics Reader; Bio-Tek Instruments, Winooski, VT, USA). Tests were performed in duplicate. All measurement (patients and control subjects) were made on the same day and under the same experimental conditions.

Dilutions of a pool of normal sera, obtained from 250 healthy volunteers, were used to generate a standard curve in each micro titer plate. This standard was arbitrarily proposed to contain EM-Ig. Results with serum samples were related to this standard and expressed as EM-Ig. The specificity of the binding of EM-Ig to EM-HMWMM was determined by competition immunoassay (Table 2). The standard curve was pre-incubated with increasing amounts of the competitors. After 1 h incubation, the standard with or without competitors was added to the EM-HWMW-coated plates and tested as described above.

Purification of human natural antibodies against endogenous mediators

Immunoadsorbent columns were prepared with antigen of interest coupled to cyanogens bromide-activated Sepharose (Pharmacia Biotech). Two milligrams of protein were used for coupling to 1.5 ml of bed volume of CNBr-activated Sepharose.

One gram of Ig in 100 ml of PBS was loaded on the immunoadsorbent column and run twice on the column at a speed of 1 ml/min at RT, followed by washing with PBS until the absorbance of the flow-through at 280 nm reached baseline values. Bound antibodies were eluted using glycine-HCl (0.1 M) buffer of pH 2.8, 2 M NaCl followed by PBS and then diethanolamine (0.1 M) buffer, pH 11; 2 M NaCl. The eluates obtained at different pH were brought to pH 7.0 and pooled. Two

| VARIABLES                      | OA       | CONTROL  |
|-------------------------------|----------|----------|
| Age (years)                   | 54 ± 8.8 | 51 ± 11.1|
| Body mass index BMI (kg/m2)   | 26 ± 2.5 | 26 ± 3   |
| Duration of OA (years)        | 13.0 ± 9.8|
| Pain (visual analog) scores   | 5.46 ± 2.15| 0.00 ± 0.00|
| Beck Depression Index         | 5.56 ± 5.69| 1.00 ± 1.86|
| Mean interval in years between baseline and follow-up scan | 2.67 |

Table 1: Baseline characteristics of study population.

HMWM was then dialyzed against Phosphate Buffered Saline (PBS), pH 7.4 at 4°C.

| %                          | SELF ANTIGEN |
|----------------------------|---------------|
| Reproducibility            | 96            | 97          | 90          | 90          |
| Sensitivity                | 26            | 13          | 23          | 8           |
| Primary kOA                | 17            | 8           | 5           | 3           |
| Specificity                | 96            | 95          | 93          | 91          |

Table 2: Reproducibility, sensitivity and specificity of detection of natural IgG antibodies in osteoarthritis of the knee.
milliliters of the flow-through fractions were allowed to run through the sorbents for two more cycles and further used as effluent fractions. Eluates and effluents were dialyzed against PBS.

Affinity of purification of natural antibodies against endogenous mediators

Immuonosorbent columns were prepared with the antigens of interest coupled with cyanogens bromide-activated Sepharose (Pharmacia Biotech) [94,95]. One milligram of protein was used for coupling with 1.5 ml of bed volume of CNBr-activated Sepharose. One gram of IVIG in 100 ml of PBS was loaded on the immunoabsorbent column and run twice on the column at a speed of 1 ml/min at RT, followed by washing with PBS until the absorbance of the flow-through at 280 nm reached baseline values. Bound antibodies were eluted using a glycine-HCl (0.1 M) buffer, pH 2.8 and 2 M NaCl followed by PBS and then diethanolamine (0.1 M) buffer, pH 11; 2 M NaCl. The eluates obtained at different pH levels were brought to pH 7.0 and pooled. Two milliliters of the flow-through fractions were allowed to run through the sorbents for two more cycles and then used as effluent fractions. Eluates and effluents were dialyzed against PBS.

Determination of total IgM and IgG levels

We used the Covalink ELISA system to determine the total IgM and IgG levels of the patients and controls. For each plate standard curves were drawn using known amounts of no conjugated human IgG and IgM.

Radiological method

Plain X-ray films were performed on the small joints of the knee in all osteoarthritis patients at baseline (n=250) and after two years (n=250). These films were examined by expert radiologists. Radiological progression was defined as an increase in the LS score from the baseline to endpoint that was greater than the median value for each patient. The Kellgren-Lawrence (KL) grade, an integer index ranging from 0 to 4, is a standard radiographic measurement of joint degradation used in diagnosing osteoarthritis of the knee [96]. Radiographic osteoarthritis can be defined simply as a KL grade of 2 or higher.

Statistical analysis

The data was analyzed on an IBM computer using SPSS. Quantitative variables were described as mean, standard deviation (SD) and range. Qualitative variables were described as number and percentage. The Chi-square test was used to compare qualitative variables between groups. The Kruskal-Wallis test was used instead of ANOVA in non-parametric data (SD>50% mean). Spearman’s correlation test was used to rank different variables against each other. Receiver Operator Characteristic Curve (ROC) was drawn to find out the best cut-off value of natural antibodies against endogenous mediators in diagnosing osteoarthritis of the knee and to test for its statistical efficacy. P-value >0.05 was considered insignificant, p<0.05 was significant and p<0.01 was highly significant.

Ethical approval

All patients and healthy controls provided informed, written consent and the study was approved by the Ethics Committee of National Institute of Rehabilitation, Mexico City, Mexico.

Results

Serological identification of natural antibodies against endogenous mediators in healthy Individuals and patients with osteoarthritis of the knee by the novel ELISA Natural self-reactive antibodies of the IgM, IgG and IgA isotype are present in the serum of healthy individuals and kOA patients. Different classes of NA (IgM, IgG, IgA) against EM (BK, ANII, VEGF, bFGF) were detected with novel ELISA protocol in the sera of kOA patients as well as in the sera of healthy individuals (Table 3). These EM represent a group of novel self-antigens which are targeted by NA from kOA patients.

In this study, we found BK-IgG expression most abundantly in testis among the kOA sera tested. No significant difference in binding of serum EM-IgA levels in kOA patients in comparison with that in control. Characterization of the functional properties of natural antibodies that recognize human endogenous mediators.

Isotypes of natural antibodies against endogenous mediators

NA belong mainly to the immunoglobulin M class and are characterized by several features, including the ability to bind self and non-self antigens, low affinity (monovalent antigenic binding to a small single epitope), high avidity (overall force that binds multivalent antibody to a macromolecule carrying multivalent epitopes), and polyreactivity (binding different epitopes). Pathogenic auto antibodies are antigen driven and belong mainly to the IgG isotype. Affinity chromatography yielded two isotypes (IgM, IgG) of EM-specific NA from the sera of healthy individuals and kOA patients. EM-IgA was not useful for the presence kOA in humans. Affinity-purified NA against EM displayed the expected characteristics and was functionally fully active.

Affinity of natural antibodies against endogenous mediators

The affinity constant of NA against self-antigens from both kOA patients and normal sera were determined in table 4. Low affinity EM-IgM was predominantly isotype of Ig which present in healthy individuals. Deficiency in the sera EM-IgM predisposes to development expression of high affinity EM-IgG in kOA patients. The secondary immune response is characterized by the rapid production of high affinity EM-IgG in kOA patients.

| The Study Group | Self Antigens |
|-----------------|---------------|
|                 | BK            | All | VEGF | bFGF |
|                 | IgG           | IgM | IgG   | IgM   | IgG | IgM | IgG | IgM |
| Patients with osteoarthritis of the knee | 689 ± 161 | 287 ± 84 | 409 ± 111 | 289 ± 99 | 699 ± 241 | 559 ± 198 | 689 ± 245 | 521 ± 222 |
|                 | 100%          | 77% | 71%   | 66%   | 59% | 79% | 35% | 61% |
| Healthy Individuals | 501 ± 98 | 407 ± 115 | 274 ± 84 | 455 ± 101 | 471 ± 189 | 366 ± 181 | 455 ± 115 | 705 ± 239 |
| Significance P value | 0.0001 | 0.0004 | 0.0005 | 0.0001 | 0.0003 | 0.005 | 0.009 | 0.007 |

Table 3: Serum expression of natural antibodies against endogenous mediators.
The cross-reactivity of affinity-purified natural antibodies against endogenous mediators to both kOA and normal sera were determined (Table 5). Serum BK-IgG was detectable in 85% of kOA patients, with 100% specificity for kOA. NA against BK and AII showed no cross reactivity to other antigens from both kOA and normal sera.

Table 4: The affinity of natural antibodies against endogenous mediators from both kOA and normal sera.

| Structurally Similar Inhibitors | % Significant Inhibition |
|-------------------------------|--------------------------|
| Angiotensin II | 0.12 | 0.01 | 100 | 100 |
| Bradykinin | 100 | 100 | 0.02 | 0.01 |
| Vasopressin | 0.003 | 0.01 | 0.07 | 0.001 |
| Demorphin | 0.001 | 0.001 | 0.003 | 0.001 |
| Beta-Endorphin | 0.007 | 0.001 | 0.001 | 0.02 |

Table 5: The cross-reactivity of affinity-purified natural antibodies against endogenous mediators from osteoarthritis of the knee and normal sera.

Table 6: Correlations between Kellgren Lawrence grading and serum markers.

| Variables | Spearman’s r | P |
|-----------|--------------|---|
| BK-IgG | 0.897 | 0.0005 |
| AII-IgG | 0.489 | 0.0027 |
| VEGF-IgG | 0.381 | 0.0045 |
| bFGF-IgG | 0.291 | 0.0001 |

Table 7: Correlations of bk-IgG titer with kellgren-lawrence grading.

| SERUM BK-IgG LEVEL, ODx1000 | CONTROL | I | II | III | IV |
|------------------------------|---------|---|----|-----|----|
| 501 ± 98 | 555 ± 131 | 635 ± 141 | 685 ± 181 | 781 ± 211 |

Table 8: Correlations between natural antibodies.

| Marker | BK-IgG | AII-IgG | VEGF-IgG | bFGF-IgG |
|--------|--------|---------|----------|----------|
| BK-IgG | -      | 0.855   | -        | 0.293    |
| AII-IgG | 0.881  | -       | 0.213    | -        |
| VEGF-IgG | 0.381  | 0.0045  | -        | 0.381    |
| bFGF-IgG | 0.211  | 0.0001  | 0.791    | -        |

Table 9: Correlations between joint space width and markers.
antigen-specific ELISA will be used in additional studies that will prove its clinical efficacy, not only for the early diagnosis osteoarthritis of the knee, but also for prognosis and the implementation of preventive steps for osteoarthritis of the knee.

Natural antibodies, which bind self-protein as endogenous mediators, are keys to the homeostasis of the immune system, particularly relating to B-lymphocytes and autoimmunity [113-116]. Serological evidence of the presence natural antibodies against endogenous mediators is an early future in osteoarthritis of the knee and not restricted to patients with end-stage disease undergoing joint replacement surgery. An understanding of the properties of natural antibodies, which characterizes their activity in relation to self-antigens, is important for binding capacity, functional activities, and immune recognition.

In this first study, we demonstrate the presence of natural polyreactive antibodies in normal human IgG that recognize bradykinin. Natural self-reactive antibodies belong to IgM, IgG and IgA isotypes. Here we found that natural IgG antibodies to bradykinin are predominantly of the IgG isotype. This may be of interest in view of previous findings indicating that natural antibodies specific for bradykinin in healthy individuals are of IgM class, while in patients osteoarthritis of the knee they are mostly IgG.

Kinins are thought to be key mediators in inflammatory joint disease. Bradykinin may exert influence on multiple players of the immune system. Bradykinin modulates the activation, proliferation, migration and effectors functions of these cells. The possible impact of bradykinin in human immune-mediated diseases could be emphasis on autoimmune neuroinflammation, osteoarthritis and infection. However, recent studies suggest a specific role of the bradykinin system in adaptive, i.e., antigen-specific immune reactions. Expression patterns of the bradykinin receptors on key cellular players within the adaptive immune system, and provide an overview of evidence so far indicating the possible involvement of the Kallikrein-kinin system in antigen-specific immune responses, including osteoarthritis conditions.

In healthy patients, chronic elevations of circulating BK-IgG or its biomarkers are predictors for increased risk in the development and progression of osteoarthritis of the knee disease. Predicting disease requires specific tests as well as a population in which a reasonable proportion of patients will develop disease [117]. The presence of BK-IgG was an important predictor for osteoarthritis of the knee.

In the present work we have shown that all IgM anti-EM antibodies and certain IgG-type anti-EM antibodies were significantly elevated in sera of patients with osteoarthritis of the knee compared with adult controls. Serum anti-EM antibody ratios were consistently low for IgM antibodies and were relatively high in the case of IgGs. In contrast to IgM, IgG gets into the inflamed synovial fluid readily, and thus, our data argue against local production of IgM type anti-EM antibodies.

The next important step was to test if the levels of BK-IgG antibodies showed any correlation with the disease activity in osteoarthritis of the knee. Intriguingly, using a multistep approach, our work has demonstrated that BK-specific IgG antibody levels show a clear inverse correlation with the activity of osteoarthritis of the knee. Thus, we suggest that BK-specific IgG is a disease-state biomarker in osteoarthritis of the knee. We found a similar relation when we analyzed the connection between disease activity and BK-IgG concentrations.

Conclusions

We showed that novel ELISA assay was able to demonstrate immune responses to each of the 4 type specific self-antigens in kOA patients. EM represents a group of novel self-antigens which are targeted by NA from kOA patients. Self-reactive NA of IgM, IgG, IgA classes are present in the sera of healthy individuals and kOA patients. This results show a specific imbalance of immunoglobulin’s content in kOA patients. Serum NA profiling is a promising approach for early detection and diagnosis of kOA. Additionally, a serum expression profiling, study identified 4 self-antigens specifically expressed in kOA patients, which were identified by affinity chromatography. High-affinity, BK-IgG has demonstrated a direct role for BK in kOA development. The isolated IgG fractions of patients suffering from kOA had higher anti-BK reactivity than those detected in normal individuals. Serum BK-IgG is a promising candidate as kOA-specific disease antibodies. Identification of novel broadly cross-reactive kOA-neutralizing NA against EM in the sera has major implications for the development of treatment, angiogenesis inhibitors, and tools to study mechanisms.

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References

1. Krasnokutsky S, Samuels J, Abramson SB (2007) Osteoarthritis in 2007. Bull NYU Hosp Jt Dis 65: 222-228.
2. Bodolyay E, Koch AE, Kim J, Szegedi G, Szelkeanecz Z (2002) Angiogenesis and chemokines in rheumatoid arthritis and other systemic inflammatory rheumatic diseases. J Cell Mol Med 6: 357-376.
3. Couture R, Harrison M, Vianna RM, Cloutier F (2001) Kinin receptors in pain and inflammation. Eur J Pharmacol 429: 161-176.
4. Rittner HL, Brack A, Stein C (2002) Pain and the immune system: friend or foe? Anaeasthesist 51: 351-356.
5. Yuan GH, Masuko-Hongo K, Kato T, Nishioka K (2003) Immunologic intervention in the pathogenesis of osteoarthritis. Arthritis Rheum 48: 602-611.
6. Cassin B, Shaw OM, Mazur M, Misso NL, Naran A, et al. (2009) Kallikreins, kininogens and kinin receptors on circulating and synovial fluid neutrophils: role in kinin generation in rheumatoid arthritis. Rheumatology (Oxford) 48: 490-496.
7. Cohen IR (2007) Biomarkers, self-antigens and the immunological homunculus. J Autoimmun 29: 246-249.
8. Fernandes JC, Martel-Pelletier J, Pelletier JP (2002) The role of cytokines in osteoarthritis pathophysiology. Biochemistry 39: 237-246.
9. György B, Tóthfalusi L, Nagy G, Plácsik M, Géher P, et al. (2008) Natural autoantibodies reactive with glycosaminoglycans in rheumatoid arthritis. Rheumatol Int 28: 573-580.
10. Jones V, Taylor PC, Jacoby RK, Wallington TB (1984) Synovial synthesis of rheumatoid factors and immune complex constituents in early arthritis. Ann Rheum Dis 43: 235-239.
11. Koch B, Locher P, Burmester GR, Mohr W, Kalden JR (1984) The tissue architecture of synovial membranes in inflammatory and non-inflammatory joint diseases. II. The localization of mononuclear cells as detected by monoclonal antibodies directed against T-lymphocyte subsets and natural killer cells. Rheumatol Int 4: 79-85.
12. Pothacharoen P, Teekachunhatean S, Louthrenno W, Yingsung W, Ong-Chai S, et al. (2006) Raised chondroitin sulfate epitopes and hyaluronan in serum from rheumatoid arthritis and osteoarthritis patients. Osteoarthritis Cartilage 14: 299-301.
13. Takahashi M, Naito K, Abe M, Sawada T, Nagano A (2004) Relationship between radiographic grading of osteoarthritis and the biochemical markers for arthritis in knee osteoarthritis. Arthritis Res Ther 6: R208-212.
14. Xiang Y, Sekine T, Nakamura H, Imajo-Ohmi S, Fukuda H, et al. (2006) Fibulin-4 is a target of autoimmune predominantly in patients with osteoarthritis. J Immunol 176: 3196-3204.
15. Ashraf S, Walsh DA (2008) Angiogenesis in osteoarthritis. Curr Opin Rheumatol 20: 573-580.
Citation: Savitskaya YA, Duarte C, Marin N, Téllez R, Alfaro A, et al. (2012) Identification of Circulating Natural Antibodies against Endogenous Mediators in the Peripheral Blood Sera of Patients with Osteoarthritis of the Knee: A New Diagnostic Frontier. J Mol Biomark Diagn 3:135. doi:10.4172/2155-9929.1000135

18. Askling J, Fored CM, Brandt L, Baeklund E, Berltsson L, et al. (2005) Risks of solid cancers in patients with rheumatoid arthritis and after treatment with tumour necrosis factor antagonists. Ann Rheum Dis 64: 1421-1426.

17. Bhoola KD, Elson CJ, Dieppe PA (1992) Kinins--key mediators in inflammatory arthritis? Br J Rheumatol 31: 509-518.

16. Bonnet CS, Walsh DA (2005) Osteoarthritis, angiogenesis and inflammation. Rheumatology (Oxford) 44: 7-16.

15. Cheng ZJ, Vapaatalo H, Mervaala E (2005) Angiotensin II and vascular inflammation. Med Sci Monit 11: RA194-205.

14. Altman R, Asch E, Bloch D, Bole G, Borenstein D, et al. (1986) Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 29: 1039-1049.

13. Williams FM (2009) Biomarkers: in combination they may do better. Arthritis Res Ther 11: 130.

12. Wollheim FA (2003) Early stages of osteoarthritis: the search for sensitive predictors. Ann Rheum Dis 62: 1031-1032.

11. Bruyere O, Collette J, Kothari M, Zaim S, White D, et al. (2006) Osteoarthritis, magnetic resonance imaging, and biochemical markers: a one year prospective study. Ann Rheum Dis 65: 1050-1054.

10. Cobankara V, Ozturk MA, Kiraz S, Ertelie I, Haznedaroglu IC, et al. (2005) Renin and angiotensin-converting enzyme (ACE) as active components of the local synovial renin-angiotensin system in rheumatoid arthritis. Rheumatol Int 25: 285-291.

9. Khakoo AY, Sidman RL, Pasqualini R, Arap W (2008) Does the renin-angiotensin system participate in regulation of human vasculogenesis and angiogenesis? Cancer Res 68: 9112-9115.

8. Stoka V, Turk V (2010) A structural network associated with the kallikrein-kinin and renin-angiotensin systems. Biol Chem 391: 443-454.

7. Sharma JN, Buchanan WW (1994) Pathogenic responses of bradykinin system in chronic inflammatory rheumatoid disease. Exp Toxicol Pathol 46: 421-433.

6. Sharma JN (1991) The role of kinin system in joint inflammatory disease. Eur J Rheumatol Inflamm 11: 30-37.

5. Lerner UH (1994) Regulation of bone metabolism by the kallikrein-kinin system, the coagulation cascade, and the acute-phase reactants. Oral Surg Oral Med Oral Pathol 78: 481-493.

4. Joseph K, Kaplan AP (2005) Formation of bradykinin: a major contributor to the innate inflammatory response. Adv Immunol 86: 159-208.

3. Koch AE, Distler O (2007) Vasculopathy and disordered angiogenesis in selected rheumatic diseases: rheumatoid arthritis and systemic sclerosis. Arthritis Res Ther: S3.

2. Rahmann MM, Bhoola KD, Elson CJ, Lemon M, Dieppe PA (1995) Identification and functional importance of plasma kallikrein in the synovial fluids of patients with rheumatoid, psoriatic, and osteoarthritis. Ann Rheum Dis 54: 345-350.

1. Uhl J, Singh S, Brophy L, Faunce D, Sawatzky DG (1992) Role of bradykinin in inflammatory arthritis: identification and functional analysis of bradykinin receptors on human synovial fibroblasts. Immunopharmacology 23: 131-138.

11. Colman RW (2006) Regulation of angiogenesis by the kallikrein-kinin system. Annu Rev Pharmacol Toxicol 46: 701-740.

10. Heffelfinger SC (2007) The renin angiotensin system in the regulation of angiogenesis. Curr Pharm Des 13: 1215-1229.

9. Clapp C, Thebault S, Zejiorski MC, Martinez De La Escalera G (2009) Peptide hormone regulation of angiogenesis. Physiol Rev 89: 1177-1215.

8. Folkman J, Klagsbrun M (1987) Angiogenic factors. Science 235: 442-447.

7. Szebenacz Z, Koch AE (2005) Endothelial cells in inflammation and angiogenesis. Curr Drug Targets Inflamm Allergy 4: 319-329.

6. Szebenacz Z, Koch AE (2004) Vascular endothelium and immune responses: implications for inflammation and angiogenesis. Rheum Dis Clin North Am 30: 97-114.

5. Avrameas S, Dighiero G, Lymberi P, Guilbert B (1983) Studies on natural antibodies and autoantibodies. Ann Immunol (Paris) 134D: 103-113.
108. Konishi E, Shoda M, Ajino N, Kondo T (2004) Development and evaluation of an enzyme-linked immunosorbent assay for quantifying antibodies to Japanese encephalitis virus nonstructural 1 protein to detect subclinical infections in vaccinated horses. J Clin Microbiol 42: 5087-5093.

109. Raiko I, Sander I, Weber DG, Rauff-Heimsoth M, Gillissen A, et al. (2010) Development of an enzyme-linked immunosorbent assay for the detection of human caietiinin in plasma and serum of mesothelioma patients. BMC Cancer 10: 242.

110. Santos-Neto JR, Mezencio JM, Chagas AT, Michereff-Filho M, Serrão JE (2010) Use of serological techniques for determination of Spodoptera frugiperda (J E Smith) predators (Lepidoptera: Noctuidae). Neotrop Entomol 39: 420-423.

111. Tanaka Y, Komori H, Mori S, Soga Y, Tsubaki T, et al. (2010) Evaluating the role of rheumatoid factors for the development of rheumatoid arthritis in a mouse model with a newly established ELISA system. Tohoku J Exp Med 220: 199-206.

112. Tate J, Ward G (2004) Interferences in immunoassay. Clin Biochem Rev 25: 105-120.

113. Jerne NK (1974) Towards a network theory of the immune system. Ann Immunol (Paris) 125C: 373-389.

114. Jerne NK (1967) [Various basic problems of current immunology]. Landarzt 43: 1526-1530.

115. Yurasov S, Nussenzweig MC (2007) Regulation of autoreactive antibodies. Curr Opin Rheumatol 19: 421-426.

116. Zhou ZH, Zhang Y, Hu YF, Wahl LM, Cisar JO, et al. (2007) The broad antibacterial activity of the natural antibody repertoire is due to polyreactive antibodies. Cell Host Microbe 1: 51-61.

117. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM (2002) How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis Rheum 46: 357-365.