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Malondialdehyde–acetaldehyde antibody concentrations in rheumatoid arthritis and other rheumatic conditions

Ted R. Mikuls[a,b,*], Michael J. Duryee[a,b], Bryant R. England[a,b], Daniel R. Anderson[c], Michelene Hearth-Holmes[a,b], Kaihong Su[d], Kaleb Michaud[b,e], Jeffrey B. Payne[b,f], Harlan Sayles[g], Carlos Hunter[b], Jacob D. McGowan[b], Lynell W. Klassen[b], Geoffrey M. Thiele[a,b]

[a] Veterans Affairs (VA) Nebraska-Western Iowa Health Care System, Omaha, NE, United States
[b] Department of Internal Medicine, Division of Rheumatology, University of Nebraska Medical Center, Omaha, NE, United States
[c] Department of Internal Medicine, Division of Cardiology, University of Nebraska Medical Center, Omaha, NE, United States
[d] Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, United States
[e] National Data Bank for Rheumatic Diseases, Wichita, KS, United States
[f] Department of Surgical Specialties, College of Dentistry, University of Nebraska Medical Center, Lincoln, NE
[g] Department of Biostatistics, College of Public Health, University of Nebraska Medical Center, Omaha, NE, United States

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ABSTRACT

Objective: To compare anti-malondialdehyde–acetaldehyde (MAA) antibody concentrations between rheumatoid arthritis (RA) patients and healthy and rheumatic disease controls.
Methods: Anti-MAA antibody (IgA, IgM, IgG) was measured using ELISA and banked serum from patients with RA (n = 284), osteoarthritis (OA, n = 330), spondyloarthropathy (SpA, n = 50), and systemic lupus erythematosus (SLE, n = 88) as well as healthy controls (n = 82). Anti-MAA antibody concentrations and the frequency of positivity were compared across groups. Multivariable linear regression analysis limited to RA and OA patients (due to sample size and data availability) was used to identify factors associated with anti-MAA antibody concentrations.
Results: Although RA patients demonstrated among the highest circulating concentrations across isotypes, only IgA anti-MAA antibody was significantly higher than all other groups ($p \leq 0.02$). Proportions (7% to 74%) of OA and SLE (less so for SpA) samples were positive for anti-MAA antibody, limiting the discriminatory capacity of anti-MAA antibody in RA (positive in 18% to 80%). In analyses limited to those with RA or OA, factors associated with higher anti-MAA antibody concentrations included RA case status, younger age (IgM), male sex (IgG), African American race (IgA, IgG) and current smoking (IgA). C-reactive protein levels and comorbidities were not associated with anti-MAA antibody concentrations.
Conclusion: With the possible exception of the IgA isotype, serum anti-MAA antibodies measured with currently available assays do not appear to adequately discriminate RA from other rheumatic conditions. With the identification of specific proteins that are MAA-modified in diseased tissues and requisite assay refinement, anti-MAA antibody holds potential promise as a biomarker in RA.

1. Introduction

Oxidative stress and its related byproducts are implicated to play a pathogenic role in the risk and progression of rheumatoid arthritis (RA) [1–4]. Formed in the process of lipid peroxidation, malondialdehyde (MDA) represents one such byproduct. MDA is a highly reactive aldehyde that is known to combine with highly reactive acetaldehyde (AA) to form malondialdehyde–acetaldehyde or the MAA adduct [5]. AA is derived not only from exogenous sources such as cigarette smoke and/or alcohol, but is produced during the spontaneous breakdown of MDA [6]. Importantly, both MDA and MAA form protein adducts, acting as potent haptens that initiate robust immune responses, not only against the MDA and MAA epitopes, but also to native carrier proteins [7]. Recently, antibody responses targeting these protein adducts have been shown to be highly characteristic of RA [8,9].

In prior investigations, we have demonstrated MAA adducts are

* Corresponding author at: Division of Rheumatology, Department of Internal Medicine, University of Nebraska Medical Center, 986270 Nebraska Medical Center, Omaha, NE 68198-6270, United States.
E-mail address: tmikuls@unmc.edu (T.R. Mikuls).

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expressed in synovial tissues in RA to a much greater magnitude than what is observed in synovial tissues taken from patients with osteoarthritis [8,9]. Importantly, these modified antigens co-localize with both citrullinated proteins and CD27+ B cells that have been implicated in autoantibody generation [8]. Moreover, anti-MAA antibody is highly enriched in RA synovial fluids compared to paired serum samples, a phenomenon not observed for other disease-specific autoantibodies. Additionally, circulating anti-MAA antibody concentrations are significantly higher in both seropositive and seronegative RA patients than in healthy controls, and among patients with RA, higher anti-MAA antibody levels are associated with more severe disease characterized by autoantibody seropositivity and the presence of extra-articular manifestations [9]. In addition to suggesting that MAA adducts might promote disease progression, these previous findings suggest that anti-MAA immune responses could serve as informative biomarkers in RA. To date, however, work examining anti-MAA antibodies as a biomarker in RA has relied on either healthy controls or a very small number of patients with osteoarthritis for comparisons [8,9]. Thus, whether higher anti-MAA antibody concentrations reflect a higher burden of systemic inflammation rather than an RA-specific disease process is unknown. This possibility is underscored by reports demonstrating increased anti-MAA antibody responses in other inflammatory disease states including cardiovascular disease, chronic airway disease, and alcoholic liver disease [6,10–14].

Therefore, in the present study, we compared anti-MAA antibody concentrations between RA patients and well-characterized diseased and healthy controls. The inclusion of appropriate diseased controls is particularly important to understand whether serum anti-MAA antibody effectively differentiates RA from other forms of inflammatory arthritis that have to date never been characterized in terms of circulating anti-MAA antibody concentrations, the same conditions that would most likely be included in a differential diagnosis and require differentiation from RA. We hypothesized that anti-MAA antibody concentrations would indeed be significantly higher in the context of RA than in other rheumatic conditions and this difference would be independent of acute phase response. Such findings would support future development of anti-MAA antibody assays for clinical use in the diagnosis of RA. This is particularly relevant as currently available autoantibody tests are negative in up to one-third of RA patients. Although we anticipated observing higher concentrations in RA patients, we also expected that a smaller proportion of diseased controls would also demonstrate robust anti-MAA antibody responses that would be associated with factors promoting oxidative stress, such as cigarette smoking.

2. Materials and methods

2.1. Patient populations

For this study, we utilized banked samples from patients with RA (n = 284), osteoarthritis (OA, n = 330), spondyloarthropathy (SpA, n = 50), and systemic lupus erythematosus (SLE, n = 88). RA and OA patients were participants in a previous case–control study examining the relationship of periodontitis with RA risk [15]. In brief, RA patients satisfied the 1987 American College of Rheumatology (ACR) classification criteria for RA [16] while OA patients were identified through medical record review with case status based on a physician diagnosis or imaging results demonstrating degenerative arthritis in the absence of inflammatory arthritis. Three cases from the original study that enrolled 287 RA patients did not have a sufficient sample available for testing anti-MAA antibody. Anti-citrullinated protein antibody (ACPA) status was determined using a commercially available second-generation anti-cyclic citrullinated peptide (CCP) antibody ELISA (Diastat; Axis-Shield Diagnostics, positivity ≥ 5 U/mL). Samples from SpA patients were obtained from the National Data Bank for Rheumatic Diseases (NDB) Biorepository [8] and included patients with rheumatologist-diagnosed psoriatic arthritis, ankylosing spondylitis, or reactive arthritis. Patients with SLE satisfied the 1997 ACR Classification criteria [17] and all were participants in the University of Nebraska Medical Center (UNMC) SLE Cohort initiated in 2010 [18,19]. In addition to samples from rheumatic disease patients, serum samples were obtained from a group of 82 healthy volunteers as previously described [10]. Rheumatology patients and healthy volunteers all provided informed written consent with the subsequent use of samples approved by the UNMC Institutional Review Board.

2.2. Anti-MAA antibody and C-reactive protein

Serum anti-MAA antibody concentrations (IgM, IgG, and IgA isotypes, ng/ml) were measured as previously described [9]. In brief, aqueous human albumin (Talecris Bio-therapeutics) was modified with 1 mM of AA and 2 mM of MDA (Sigma-Aldrich) to form the 1,4 dihydropyridine albumin MAA adduct. Native albumin and MAA-modified albumin were coated on 96-well ELISA plates at a concentration of 2 μg/well and incubated overnight at 4 °C [20]. To create a standard curve, known concentrations of human IgA, IgM, or IgG were also coated and incubated overnight. Plates were washed with PBS-tween using a 405 TS Microplate washer (BioTek), blocked with 2% casein, and incubated with serum sample for one hour. Reactivity to anti-MAA antibodies (ng/mL) was determined using secondary horseradish peroxidase (HRP)-conjugated goat anti-human antibody specific for IgM (5 μ Fc fragment–specific), IgG (Fcγ-specific), or IgA (α-chain–specific) (Jackson ImmunoResearch) and developed using tetramethylbenzidine (TMB) substrate. Absorbance was determined at 450 nM using an Epoch Plate reader (BioTek) and analyzed using Gene 5 Software (BioTek). Consistent with the 1987 ACR classification criteria for RA, referent to defining a positive laboratory threshold for rheumatoid factor (RF) concentrations [16], anti-MAA antibody positivity was defined as values exceeding the 95th percentile observed in healthy volunteers. High sensitivity C-reactive protein (hs-CRP, mg/L) was determined in all study samples using nephelometry (Siemens Healthcare Diagnostics, Munich, Germany).

2.3. Statistical analysis

Results are expressed as means ± standard deviation (SD) or medians with corresponding interquartile ranges (IQR), as appropriate. Anti-MAA antibody concentrations were compared for each isotype across patient/control groups using the non-parametric Kruskal–Wallis rank test with Dunn's post-hoc test for pairwise comparisons. Given the broad range in raw values observed, anti-MAA antibody concentrations were log-transformed for graphical purposes. Additional multivariable linear regression analyses were conducted to examine whether group differences (RA vs. OA, SLE, SpA, or healthy control) in IgA anti-MAA antibody concentrations were independent of age, sex, race, and hs-CRP concentration.

The proportions of individuals who were positive for each anti-MAA antibody isotype, defined as those with serum concentrations exceeding the 95th percentile in healthy volunteers, were compared using chi-square tests. To examine whether anti-MAA antibody values effectively discriminate RA cases from other controls, we calculated the area under the curve (AUC) for receiver operator curves (ROCs) for each isotype. Three ROCs with different comparison groups were generated, one limited to healthy controls, one limited to diseased controls, and another including both healthy volunteers and diseased controls in the comparison group.

To identify other factors associated with anti-MAA antibody concentration beyond disease status, we performed multivariable linear regression analysis limited to RA and OA patients. By using a multivariable approach, we were able to simultaneously assess the contribution of several additional confounders to anti-MAA antibody expression. We limited these analyses to RA and OA because these were