Autoantibodies to GPI and creatine kinase in RA

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Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease that primarily affects the joints. The trigger that activates autoreactive T and B cells in RA patients is still unknown[1].

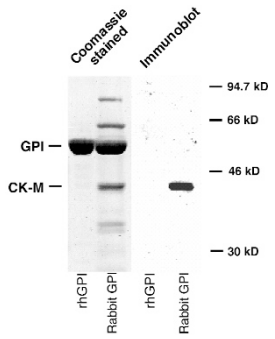

Figure 1. CK-M is contained in the commercial GPI preparation and recognized by sera from RA patients. (Left panel) Coomassie blue–stained SDS-PAGE of recombinant human GPI (rhGPI) and purified rabbit GPI. The different GPI preparations were blotted on nitrocellulose membranes and assayed with several different patient sera (1:200). Some of the sera that recognized the commercial GPI preparation when tested by ELISA recognized one of the contaminating proteins. (Right panel) One characteristic example is shown.

The glycolytic enzyme glucose-6-phosphate-isomerase (GPI) has been identified as the antigen recognized by arthritogenic antibodies in a spontaneously developing murine model of arthritis that has several of the features of human RA[2]. It is, therefore, of interest to determine whether GPI is also relevant to human arthritides, such as RA. Thus, we expressed recombinant human GPI fused to a histidine tag in *Escherichia coli*. The expressed protein had the expected enzymatic activity (data not shown) and was used to examine serial dilutions of sera from patients with RA, other rheumatic diseases (including ankylosing spondylitis, vasculitis and connective tissue disease) or healthy controls for the presence of antibodies to GPI. At a dilution of 1:50, only 2/61 sera from RA patients bound recombinant human GPI (**Web Fig. 1** online). This contrasts with an article by Schaller *et al.* published in *Nature Immunology* in which 64% of sera (diluted 1:50) from RA patients had detectable IgG to a commercial GPI preparation, which was isolated from rabbit muscle[3]. When we used the commercial GPI preparation for ELISA analysis, only a few samples contained detectable anti-GPI at dilutions >1:50 (data not shown). At the 1:50 dilution, we detected increased antibody titers in 15/61 RA serum samples, 9/21 serum samples from patients with other rheumatic diseases and 1/32 serum samples from healthy donors (**Web Fig. 2** online).

To identify the reasons for this markedly different reactivity, we separated the two different GPI preparations by SDS-PAGE and stained the gels with Coomassie blue. The commercially available rabbit-GPI preparation contained several bands in addition to the band at 55 kD, the expected molecular weight of GPI. Immunoblotting showed that several RA patient sera recognized one of the contaminating bands, which was at ~40 kD (**Fig. 1**). We used peptide mass fingerprinting by matrix-assisted laser desorption-ionization mass spectrometry (MALDI-MS)[4] to determine the sequence of this protein. We found that 21/24 peaks with a sequence coverage of 64% identified this protein as the M chain of rabbit creatine kinase (CK-M, **Web Fig. 3** online). To test whether the ELISA reactivity observed with the commercial rabbit-GPI preparation was indeed directed against CK-M, we examined RA patient and control sera for reactivity with CK-M: 10/61 RA sera bound CK-M. Among those ten sera were four that also recognized the commercial GPI preparation (**Web Fig. 4** online). Thus, a high proportion of the serological response to the commercially available GPI preparation from rabbit muscle was directed against contaminants such as CK-M. In summary, antibody responses against CK-M and GPI can be detected at low serum dilution in some RA patients. Their pathogenic and diagnostic relevance in human disease is the subject of ongoing research.

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Note: Supplementary information is available on the Nature Immunology *website.*

Few human autoimmune sera detect GPI

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RA is a common disease with a complex etiology and pathogenesis. Many different animal models are used to study RA. With the use of one such model of a spontaneously developing arthritis, serum antibodies and transgenic T cells were shown to react with the ubiquitously expressed glycolytic enzyme GPI. Purified anti-GPI, as well as sera from sick mice, transmitted RA into naïve mice, even in the absence of B and T cells[3,4]. In an article published in *Nature Immunology* by Schaller *et al.*[5], the authors suggested that GPI might be the autoantigen in human RA as well. They also claimed that GPI could be used to diagnose 64% of the RA cases. In particular, sera from other autoimmune diseases, such as Lyme disease and Sjögren's syndrome, did not react with GPI. Thus, this would represent the first molecular tool for diagnosing RA to this extent.

We started a parallel analysis of human sera from patients with RA, Sjögren's syndrome, systemic lupus erythematosus (SLE), overlap syndrome, polyarthritis, crest syndrome, collagenosis, Wegener's granulomatosis and various other autoimmune diseases (n=462), but—with the use of recombinant HPLC-purified human GPI[6] and three different secondary antibodies to detect bound human IgG in an ELISA assay—detected reactivity to GPI in only a few cases (**Fig. 1**). We analyzed one set of sera, which included