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Nucleic acid-stimulated antigen-presenting cells trigger T cells to induce disease in a rat transfer model of inflammatory arthritis

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ABSTRACT

Autoimmune responses to heterogeneous nuclear ribonucleproteins (hnRNP) occur in many systemic autoimmune diseases, particularly in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus. In RA, humoral and/or cellular autoimmunity to hnRNP-A2/B1 is the most prominent anti-nuclear reactivity, being detectable in more than 50% of patients. However, its pathogenic role has not been fully elucidated yet. Here, we report that splenocytes from rats with pristane-induced arthritis transfer disease after *in vitro* restimulation with hnRNP-A/B antigens. Remarkably, disease transfer can be blocked by nuclease treatment of hnRNPs and is also achieved with splenocytes stimulated with hnRNP-A/B associated DNA or RNA oligonucleotides (ON) alone. Induction of proinflammatory cytokines in splenocytes stimulated with hnRNP-A/Bs or ONs involves Toll-like receptors (TLR) 7 and 9 but not TLR3. Furthermore, although T cells are the main mediators of disease transfer they require restimulation with TLR-activated antigen-presenting cells such as macrophages in order to become arthritogenic. Thus, the autoantigenic properties of hnRNPs appear to be mediated by their associated nucleic acids binding to TLR7 and 9. Our data explain the specific selection of hnRNP-A2/B1 as autoantigen in RA and reveal the requirement of interaction between innate and adaptive immunity to initiate and drive inflammation in autoimmune arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoinflammatory condition affecting approximately 1% of the world's population and is characterized by a destructive inflammation of the joints, leading to progressive disability and reduced life expectancy. The RA synovial membrane is infiltrated by immune cells, predominantly macrophages, neutrophils and T cells, resulting in the chronic overproduction of proinflammatory cytokines and proteolytic enzymes that cause cartilage and bone degradation [1].

Genetic and serologic evidence in both RA and experimental models of arthritis suggests a pathogenic role for both innate and adaptive autoimmune processes. Toll-like receptors (TLRs), a family of evolutionarily conserved pattern recognition receptors, form a bridge between the innate and adaptive arms of the immune

Abbreviatons: AUC, area under the curve; CIA, collagen-induced arthritis; CS, complementary sequence; DOTAP, N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; gtp, glucose transporter; hnRNP, heterogeneous nuclear ribonucleoprotein; HPRT, hypoxanthine phosphoribosyl transferase; IQ, imiquimod; ODN, oligodesoxyribonucleotide; ON, oligonucleotide; ORN, oligoribonucleotide; pDC, plasmacytoid dendritic cell; PIA, pristane-induced arthritis; PKM, pyruvate kinase mRNA; RA, rheumatoid arthritis; RASF, rheumatoid arthritis synovial fibroblast; SLE, systemic lupus erythematosus; TLR, Toll-like receptor; tr, telomeric repeat; TRAP, tartrate-resistant acid phosphatase.

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system, and have been suggested to be important factors in the development of RA. The 10 human TLRs identified to date can be classified into two distinct groups based on cellular distribution and ligand repertoire. Cell surface-expressed TLRs 1, 2, 4, 5, and 6 recognize ligands of bacterial and fungal origin, whereas TLRs 3, 7, 8, and 9 are expressed predominantly in the endosomal compartment and detect mainly viral nucleic acids. In addition to microbial products, TLRs are activated by a number of endogenous molecules that can be produced during tissue damage and have been detected in inflamed joints [2]. Examples of such molecules include heat shock proteins, hyaluronan, and high-mobility group box protein-1, but also endogenous RNA derived from necrotic cells that may activate cultured RA synovial fibroblasts (RASF) via TLR3 [3]. Since the expression of TLRs 2, 3, 4, and 7 has been reported in human RA tissue [4–6] the concept of endogenous ligand-driven activation of TLR signalling has raised interest in these receptors as potential candidates in the induction and/or maintenance of chronic inflammatory conditions such as RA.

Autoimmune reactivity to antigens that are associated with nucleic acids is a hallmark of many autoimmune disorders and is commonly found in diseases such as systemic lupus erythematosus (SLE), progressive systemic sclerosis, primary Sjögren's syndrome, and, though to a lesser extent, also RA. In RA, the most specific anti-nuclear reactivity is directed towards heterogeneous nuclear ribonucleoprotein (hnRNP)-A2/B1, an abundant RNA- and DNA-binding protein that shows a predominantly nuclear localization and exerts multiple functions including the processing, transport and translation of mRNA [7,8]. HnRNP-A2/B1 is targeted by autoantibodies and T cells of patients with RA, SLE, and mixed connective tissue disease [9–13]. Interestingly however, B cell epitope recognition was found to differ among the diseases [14,15] and remarkable differences were observed between RA and SLE patients with respect to T cell reactivity [10,11].

In a recent study we discovered hnRNP-A2/B1 to be an early B- and T cell-autoantigen in pristane-induced arthritis (PIA), a rat model of inflammatory arthritis. PIA closely mimics RA as it fulfills many of the clinical criteria of RA, including a symmetrical involvement of peripheral joints and the presence of rheumatoid factor, the destruction of cartilage and bone, and a chronic disease course [16]. Pronounced T cell reactivity against hnRNP-A2/B1 was seen already one week before disease onset while autoantibodies were detectable around onset with titres increasing during the acute phase, returning to normal levels thereafter. T cells reactive to hnRNP-A2/B1 were CD4 positive and responded to stimulation by hnRNP-A2/B1 with the expression of high amounts of IFN-γ and IL-17 [17,18]. Unexpectedly, hnRNP-A2/B1 was also able to stimulate secretion of inflammatory cytokines in monocytes and macrophages derived from naïve (non pristane-primed) animals, that was dependent on the TLR adaptor molecule MyD88 [17].

In the current study we demonstrate that splenocytes from pristane-primed rats restimulated with hnRNP-A2/B1 or other closely related hnRNP proteins induce a highly inflammatory and erosive arthritis in naïve recipient rats that clinically and histologically resembles arthritis triggered by direct injection of pristane. Importantly, disease transfer can be inhibited by treatment of hnRNP-A/B with nucleases or chloroquine, an inhibitor of endosome acidification, and can also be achieved by stimulation of splenocytes with certain hnRNP-associated RNA- or DNA sequences alone, revealing that activation of endosomal TLRs is essential for disease transfer. Although arthritis is induced by T cells, transfer requires coincubation with TLR7 and/or TLR9-expressing antigen-presenting cells (APC). Thus, the arthritogenic properties of hnRNPs are based on cooperation between innate and adaptive immunity. Interestingly, macrophages appear to have higher capacities than dendritic cells for the induction of arthritogenicity in T cells. Our data significantly support the hypothesis of a pathogenic role of hnRNP-A/B proteins in erosive arthritis and suggest that autoimmunity to nucleic acid-associated autoantigens has the potential to contribute crucially to the development of diseases such as PIA and RA.

2. Materials & methods

2.1. Animals

The immune response to hnRNP-A2/B1 has been previously shown to be particularly associated with the $RT1^f$ (MHC) haplotype on the DA background (DA.1F) [17]. All animals were therefore conducted with congenic DA.1F rats (originating from Zentralinstitut für Versuchstierzucht, Hannover, Germany). Rats were bred and maintained under Specific Pathogen Free conditions in the animal facility of the Institute of Biomedical Research, Medical University of Vienna, Austria. Rats were housed in groups of two to three in plastic cages with 12 h light/dark-cycles. Standard rodent chow and water was provided *ad libitum*. Experiments were performed on rats frequency-matched for age and sex and in mixed-treatment cages to compensate for cage effects. All experiments were approved by the local ethical committee.

2.2. Transfer of pristane-induced arthritis

At day 14 after intradermal injection of 500 μl pristane oil (2, 6, 10, 4-tetramethylpentadecane, Sigma–Aldrich, St.Louis, USA) at the base of the tail rats were killed, and spleens were removed. Erythroctyes were lysed with red blood cell lysing buffer (Sigma–Aldrich) and cells were washed and passed through 70 μm filters. Different cell types were then isolated or total spleen cells were reactivated *in vitro* by incubation with natural hnRNP-A2/B1, recombinant hnRNP-A1, -A2/B1, or -A3 (20 μg/ml), Con A (3 μg/ml, Sigma–Aldrich), Imiquimod (5 μM, Cayla InvivoGen, Toulouse, France), poly(I:C) (8 μg/ml, Cayla InvivoGen), or DNA and RNA ONs (0.5 μM, VBC Biotech, Vienna, Austria) in DMEM supplemented with FCS (5%), HEPES (2.4 mg/ml), 2-ME (3.9 μg/ml), and penicillin-streptomycin (104 IU/ml penicillin, 10 mg/ml streptamycin; Invitrogen Life Technologies) termed complete DMEM. The liposomal transfection agent DOTAP (Roche, Basel, Switzerland) was used at 20 μg/ml to enhance stability and intracellular uptake of nucleic acids and hnRNPs. In some experiments, 5 μg/ml RNase and/or DNAse (both from Boehringer Mannheim, Germany), 5 μM chloroquine (Sigma–Aldrich) or 10 μg/ml of anti-RT1B and anti-RT1D mAbs (both from Acris, Herford, Germany) were added to the culture. After 48 h of incubation at 37 °C, cells were washed and resuspended in PBS-D. Naive recipient rats were then irradiated at 600 rad at the Department of Radiation oncology of the Medical university of Vienna using a Cobalt-60 source, anesthetized with isofluorane and injected subretinally with different numbers of cells as indicated in the text.

2.3. Isolation of cell types from rat and human

Different kinds of APCs, B cells, and T cells were purified from rat spleens using a combination of adherence to plastic, magnetic activated cell sorting (MACS) (Miltenyi Biotec, Bergisch-Gladbach, Germany) and fluorescence activated cell sorting (FACS). Surface markers used for distinguishing cell types are listed in Suppl. Table 1. Purity was generally >95%. For enrichment of macrophages, splenocytes were incubated before cell sorting for 2 h at a density of 5×10^6/ml in complete DMEM in petri culture dishes. Non-adherent cells were removed by extensive washing with