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Species-specific immune responses generated by histidyl-tRNA synthetase immunization are associated with muscle and lung inflammation

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Abstract

Evidence implicating histidyl-tRNA synthetase (Jo-1) in the pathogenesis of the anti-synthetase syndrome includes established genetic associations linking the reproducible phenotype of muscle inflammation and interstitial lung disease with autoantibodies recognizing Jo-1. To better address the role of Jo-1-directed B and T cell responses in the context of different genetic backgrounds, we employed Jo-1 protein immunization of C57BL/6 and NOD congenic mice. Detailed analysis of early antibody responses following inoculation with human or murine Jo-1 demonstrates remarkable species-specifity, with limited cross recognition of Jo-1 from the opposite species. Complementing these results, immunization with purified peptides derived from murine Jo-1 generates B and T cells targeting species-specific epitopes contained within the amino terminal 120 amino acids of murine Jo-1. The eventual spreading of B cell epitopes that uniformly occurs 8 weeks post immunization with murine Jo-1 provides additional evidence of an immune response mediated by autoreactive, Jo-1-specific T cells. Corresponding to this self-reactivity, mice immunized with *murine* Jo-1 develop a striking combination of muscle and lung inflammation that replicates features of the human anti-synthetase syndrome.

Keywords: Autoantibodies; Autoantigens; Inflammatory myopathy; Lung inflammation; Animal models

1. Introduction

Polymyositis (PM) and dermatomyositis (DM) represent autoimmune diseases in which muscle is inappropriately targeted for immune-mediated destruction. Both of these inflammatory myopathies can produce systemic complications that include vasculopathy (Raynaud's phenomenon), arthritis, dysphagia, cardiac dysfunction, and interstitial lung disease [1].

Abbreviations: PM, polymyositis; DM, dermatomyositis; Jo-1, histidyl-tRNA synthetase; HA/MBP, amino terminal 151 amino acid fragment of human Jo-1 fused to MBP; MA/MBP, amino terminal 151 amino acid fragment of murine Jo-1 fused to MBP; MBP, maltose binding protein; CFA, complete Freund's adjuvant.

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Yet, this clinical overlap contrasts with histologic findings that reflect potential differences in the immunopathogenesis of these two entities. While DM is characterized by perivascular B cells, CD4 + T cells, and membrane attack complex formation (terminal complement components C5–C9), the immunohistologic hallmarks of PM include endomysial T cells (CD4+ and CD8+) and the absence of complement deposition [2–4]. Thus, DM appears to result from immune complex-triggered vascular/perivascular inflammation, whereas PM stems from T cell-mediated cytolysis/dysfunction of muscle cells [5].

Despite the wealth of data supporting a primary role for T cells in the pathogenesis of PM, the antigenic trigger(s) for T cell-mediated autoimmunity in this disorder remain undefined. An important clue, however, lies in the clinical specificity of patient subsets defined by autoantibodies directed against antigens such as histidyl-tRNA synthetase (Jo-1) [6]. Antibodies recognizing a number of different tRNA synthetases, of which anti-Jo-1 are the most common, serve as the serologic hallmark of the anti-synthetase syndrome that consists of myositis, interstitial lung disease, arthritis, and fever [6]. Of note, the lack of serologic overlap between these subsets indicates that antibody formation reflects a specific, antigen-driven process rather than a bystander response to muscle injury (in which case multiple antibody specificities would be expected to co-exist).

Although previous work including histomorphologic studies does not support a direct role for anti-Jo-1 antibodies in the pathogenesis of PM, several additional pieces of evidence from extensive analysis of the B cell response in this disease implicate Jo-1 as a pathogenic autoantigen. First, antibody titers against Jo-1 correlate with disease activity in these patients [7–9]. Second, epitope mapping studies have demonstrated an increasingly complex, polyclonal anti-Jo-1 antibody response that recognizes several different portions of Jo-1, disfavoring a simple molecular mimicry hypothesis in which cross-reactive antibodies directed against a different target molecule should only bind a single epitope of Jo-1 [8,10–12]. Third, and most significantly, serial binding studies demonstrate affinity maturation of the Jo-1 antibody response over time [10]. Because antigen-specific B cell responses are ultimately T cell driven [13], this collective data strongly suggests that Jo-1 serves as a triggering antigen for CD4 + T cells that provide help for B cells as well as cytolytic T cells directed against myocytes expressing Jo-1.

Consistent with this hypothesis, we have previously demonstrated Jo-1-specific T cells in the peripheral blood of Jo-1 antibody-positive patients [14]. However, because T cells targeting Jo-1 can also be found in individuals without disease [14], a direct link between such T cells and myocytotoxicity is currently lacking. Establishing appropriate *in vitro* and *in vivo* models to analyze Jo-1-specific T cell clones is therefore necessary to determine whether antibodies targeting Jo-1 merely represent markers of disease or reflect *pathogenic, antigen-specific* T cell responses. Unfortunately, most of the existing animal models of myositis have provided little more than general insight concerning candidate autoantigens, underscoring the need for newer systems that explore the basis of the clonal/oligoclonal T cell expansion found in diseased muscle of human PM patients [15,16].

While various models of other autoimmune diseases show that TCR (T cell receptor) repertoire is a key component in the breakdown of tolerance to self-antigen, disease expression ultimately depends on factors that influence T cell effector *function*. Genetic studies in congenic strains of NOD mice provide additional insight concerning the relative contributions of T cell structure (i.e., TCR repertoire) and function to tolerance breakdown in autoimmune diabetes. Pertinent to model development for Jo-1-induced myositis, this and other work suggests that both MHC and non-MHC loci of different genetic strains are critical variables contributing to any potential disease phenotype [17]. NOD congenic variants therefore represent an ideal system for the analysis of Jo-1 immunization protocols and the impact of genetic background on disease expression, particularly given the underlying ‘‘defects’’ in tolerance as well as generalized autoimmune diathesis linked to the parental NOD genotype [18–23]. In the context of these different genetic backgrounds, the following studies demonstrate that B cell and, by extension, T cell responses to Jo-1 immunization are remarkably species-specific despite >95% homology between the murine and human versions of this protein. Based on the specificity of this response, we have used *murine* Jo-1 to generate autoreactive B and T cells against native Jo-1 that ultimately produce muscle and lung inflammation paralleling the human anti-synthetase syndrome.

2. Materials and methods

2.1. Antigen preparation

Recombinant fragments as well as full length versions of both human and murine histidyl-tRNA synthetase (Jo-1) were generated as maltose binding protein (MBP) fusion proteins following subcloning of appropriate sequences into the bacterial expression vector pMALc2 (New England Biolabs, Ipswich, MA). *In situ* mutagenesis (Stratagene, La Jolla, CA) with insertion of a stop codon after base pair 453 yielded constructs encoding 151 amino acid fragments of both human (HA) and murine (MA) Jo-1. While the human sequences were derived from a cDNA library of a healthy control subject, mouse Jo-1 cDNA was obtained via RT-PCR amplification of C57BL/6 myocyte RNA (courtesy of C.C. Liu). Expressed proteins were purified with amylose resin per the manufacturer's protocol (New England Biolabs, Ipswich, MA), filter sterilized, and then subjected to additional column purification for endotoxin removal (Profos AG, Regensburg, Germany) prior to use in proliferation assays. As previously described [14], full length versions of Jo-1 were cleaved with Factor Xa (New England Biolabs, Ipswich, MA) to release the MBP moiety and further purified using ion exchange chromatography.

Overlapping peptides (18–20 mers) comprising the amino terminal 120 amino acids of murine Jo-1 were synthesized and HPLC purified by the University of Pittsburgh Molecular Medicine Institute using Fmoc chemistry.