Histidyl–tRNA Synthetase and Asparaginyl–tRNA Synthetase, Autoantigens in Myositis, Activate Chemokine Receptors on T Lymphocytes and Immature Dendritic Cells

O.M. Zack Howard,¹ Hui Fang Dong,² De Yang,¹ Nina Raben,³ Kanneboyina Nagaraju,⁴ Antony Rosen,³ Livia Casciola-Rosen,⁴ Michael Härtlein,⁵ Michael Kron,⁶ David Yang,⁷ Kwabena Yiadom,⁷ Sunita Dwivedi,³ Paul H. Plotz,³ and Joost J. Oppenheim¹

¹National Cancer Institute, Center for Cancer Research, Laboratory of Molecular Immunoregulation, and 2Intramural Research Support Program, SAIC Frederick, NCI-Frederick, Frederick, MD 21702
²Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892
³Department of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD 21218
⁴Institute Max von Laue, Paul Langevin, and ⁵Department of Medicine, Michigan State University, East Lansing, MI 48823
⁶Department of Biochemistry, Georgetown University, Washington, DC 20057

Abstract

Autoantibodies to histidyl–tRNA synthetase (HisRS) or to alanyl–, asparaginyl–, glycyl–, isoleucyl–, or threonyl–tRNA synthetase occur in ~25% of patients with polymyositis or dermatomyositis. We tested the ability of several aminoacyl–tRNA synthetases to induce leukocyte migration. HisRS induced CD4⁺ and CD8⁺ lymphocytes, interleukin (IL)–2–activated monocytes, and immature dendritic cells (iDCs) to migrate, but not neutrophils, mature DCs, or unstimulated monocytes. An NH₂-terminal domain, 1–48 HisRS, was chemotactic for lymphocytes and activated monocytes, whereas a deletion mutant, HisRS-M, was inactive. HisRS selectively activated CC chemokine receptor (CCR)5-transfected HEK-293 cells, inducing migration by interacting with extracellular domain three. Furthermore, monoclonal anti-CCR5 blocked HisRS-induced chemotaxis and conversely, HisRS blocked anti-CCR5 binding. Asparaginyl–tRNA synthetase induced migration of lymphocytes, activated monocytes, iDCs, and CCR3-transfected HEK-293 cells. Seryl–tRNA synthetase induced migration of CCR3-transfected cells but not iDCs. Non-autoantigenic aspartyl–tRNA and lysyl–tRNA synthetases were not chemotactic. Thus, autoantigenic aminoacyl–tRNA synthetases, perhaps liberated from damaged muscle cells, may perpetuate the development of myositis by recruiting mononuclear cells that induce innate and adaptive immune responses. Therefore, the selection of a self-molecule as a target for an autoantibody response may be a consequence of the proinflammatory properties of the molecule itself.

Key words: myopathy • chemokine receptor • aminoacyl–tRNA synthetase • autoantibody • autoimmunity

Introduction

In the human idiopathic inflammatory myopathies, polymyositis, and dermatomyositis, patients experience debilitating muscle weakness associated with an inflammatory cellular infiltrate. Autoantibodies are common, and among them are a number that are specific for myositis (1, 2). The most common of these are directed against one or another of the aminoacyl–tRNA synthetases. The most frequent specificity (anti-Jo-1) is for histidyl–tRNA synthetase (HisRS),* found...
in ~15 to 25% of patients. A smaller percentage has autoantibodies directed to alanyl–, asparaginyl–, glycyl–, isoleucyl–, or threonyl–tRNA synthetases. Autoantibodies to both tryptophanyl– and seryl–tRNA synthetases have been reported in a few patients with systemic lupus erythematosus or rheumatoid arthritis, but not myositis (3). The remaining aminoacyl-tRNA synthetases have not been identified to be the targets of autoantibodies in those or other illnesses, with the exception that autoantibodies to LysRS occur in some patients with anti-IleRS, presumably by epitope spreading since both proteins are part of the multi-aminoacyl–tRNA synthetase complex. The epitopes recognized by anti-Jo-1 are not identical in all patients (4), but the predominant antigenic domain is the α-helical NH₂ terminus that is a coiled-coil (5, 6). How these intracellular components of protein translation participate in the development and progression of idiopathic myopathies is not understood.

The reasons for the generation of the autoantibody repertoire directed against a restricted set of autoantigens in myositis or indeed in any autoimmune disease, have been the object of extensive, but largely inconclusive speculation. The explanations that postulate molecular mimicry of a self component by a microorganism seem increasingly unlikely (7, 8), and a generalized up-regulation of immunity or down-regulation of tolerance lack the power to explain the limited repertoire that is observed in human and murine autoimmune disease. Recent observations by Dohlm an on the frequent occurrence of coiled-coil structures in autoantigens (6, 9, 10), by Casciola-Rosen and Rosen on the cleavability of autoantigens by caspases or granzyme B and their localization in apoptotic blebs (11–14), and by Utz, Anderson, van Vennroj, Tan, and Mamula and their colleagues on modifications of proteins that increase their antigenicity have pointed to the important or even essential role of the biochemical structure of the autoantigens in determining their selection as targets (15–18).

It has been reported that human tyrosyl–tRNA synthetase (TyrRS), not a known autoantigen, has both chemoattractant and leukocyte activating activities when proteolytically cleaved (19, 20). Proteolysis of TyrRS by neutrophil elastase produces an NH₂-terminal domain composed of amino acids 1–364 and a COOH-terminal domain composed of amino acids 365–528. The NH₂-terminal domain of TyrRS binds to CXC chemokine receptor (CXCR)1 and induces neutrophil chemotaxis, whereas the COOH-terminal domain behaves like endothelial–monocyte activating polypeptide II (EMAPII), inducing myeloperoxidase and TNF production as well as monocyte chemotaxis. In addition, functional homologues of EMAPII have been identified in other aminoacyl–tRNA synthetases (21). These findings suggested to us the possibility that proinflammatory properties, specifically the chemoattractant properties, of other aminoacyl–tRNA synthetases might contribute to their choice as targets in myositis.

Both infiltrating lymphocytes and muscle cells in the muscle tissue of patients express costimulatory markers (22) and produce several proinflammatory cytokines. Several reports have shown that elevated levels of selected chemo...
membrane precoated with 50 mM glutamine, 25 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 ng/ml of rhGM-CSF, and 50 ng/ml of rhIL-4 at 37°C in a humidified CO₂ (5%) incubator (33). Half of the medium was replaced on days 3 and 5 with fresh G4 medium. On day 7, the cells in suspension were collected and used as iDCs. As confirmed by flow cytometry analysis, iDCs were CD1a⁺, CD14⁺, CD40low, CD83⁺, CD86low, HLA-DRmedium. Mature dendritic cells (DCs) were generated by treating the iDCs with 50 ng/ml of rhTNF-α over the chemokines. In the case of lymphocyte chemotaxis, five micrometer polycarbonate membranes were placed in the lower wells of a chemotaxis chamber (Neuroprobe). To observe chemotaxis of primary leukocytes, five micrometer polycarbonate membranes were placed in the lower wells of a chemotaxis chamber (Neuroprobe). To observe chemotaxis of primary leukocytes, five micrometer polycarbonate membranes were placed in the lower wells of a chemotaxis chamber (Neuroprobe). To observe chemotaxis of primary leukocytes, five micrometer polycarbonate membranes were placed in the lower wells of a chemotaxis chamber (Neuroprobe). To observe chemotaxis of primary leukocytes, five micrometer polycarbonate membranes were placed in the lower wells of a chemotaxis chamber (Neuroprobe). To observe chemotaxis of primary leukocytes, five micrometer polycarbonate membranes were placed in the lower wells of a chemotaxis chamber (Neuroprobe). To observe chemotaxis of primary leukocytes, five micrometer polycarbonate membranes were placed in the lower wells of a chemotaxis chamber (Neuroprobe).

Chemotaxis. Cells were resuspended in binding media (RPMI 1640 containing 1% bovine serum albumin, 25 mM HEPES, pH 8.0) at 1–5 × 10⁶ cells/ml. Chemokines diluted in binding medium were placed in the lower wells of a chemotaxis chamber (Neuroprobe). To observe chemotaxis of primary leukocytes, five micrometer polycarbonate membranes were placed over the chemokines. In the case of lymphocyte chemotaxis, membranes were precoated with 50 μg/ml of fibronectin. To analyze the response of HEK transfectants, a 10-μm polycarbonate membrane precoated with 50 μg/ml rat tail collagen type1 (Collaborative Biomedical Products) at 37°C for 2 h was placed over the chemotaxitants. After the micro-chemotaxis chamber was assembled, 50 μl of cells were placed in the upper wells. If receptor blocking studies were being performed, 20 μg/ml of a receptor blocking monoclonal antibody or an isotype control (45531; R&D Systems) was added to the cell suspension 30 min before the cells were added to the upper wells. The filled chemotaxis chambers were incubated in a humidified CO₂ incubator for 60 min for neutrophils, 90 min for monocytes, 3 h for lymphocytes, or 5 h for HEK transfectants. After incubation the membranes were removed from the chemotaxis chamber assembly followed by gently removing cells from the upper side of the membrane. The cells on the lower side of the membrane were stained using Rapid Stain (Richard-Allen Scientific). The migrated cells were counted by computer using the BIOQUANT 98 program (R&M Biometrics). The number of cells migrating through the membrane in a high power field was determined for medium alone, chemokine-treated, and untreated cells. The average number of cells per high powered field (200× magnification) was determined for a given chemokine concentration and the standard deviation reported in the error bars. Unpaired Student t tests were performed between the media control and given chemokine concentrations. Chemotactic index (C.I.) was calculated as follows: (average number of migrating cells in sample/average number of cells in medium control). C.I. was considered significant if P < 0.001 compared with medium control. All experiments were repeated at least three times.

Binding Studies. Binding assays were performed in triplicate by adding increasing amounts of unlabeled competitor and constant ¹²⁵I radiolabeled chemokine, 0.2 ng/assay (MIP 1 β-299; New England Nuclear) to individual 1.5 ml microfuge tubes. 200 μl/samples of cells (2 × 10⁶ cells/ml) suspended in binding media were added to the tubes and mixed by continuous rotation at room temperature for 45 min. After incubation the cells were centrifuged through a 10% sucrose/PBS cushion and the cell-associated radioactivity was measured using a 1272 Wallac gamma counter. A minimum of three independent binding assays were performed in triplicate for each cell type and radiolabeled chemokine.

Immunohistochemistry for CCR3 and CCR5. Immunohistochemistry was performed as described previously (22). Briefly, frozen muscle tissues from eight myositis patients were acetonite fixed and blocked first with peroxidase blocking solution (Dako) and then with serum containing 1:1 goat serum and pooled human serum. The sections were incubated overnight at 4°C with a 1:50 dilution of primary monoclonal anti-human CCR5 or anti-human CCR3 (R&D Systems) and with anti-mouse horseradish peroxidase (HRP; Dako) for 1 h at room temperature. The tissues were developed with DAB chromogen (Dako) and counterstained with hematoxylin. Isotype matched (IgG2b) controls were used in place of primary antibodies to confirm the specificity.

Fluorescence-activated Cell Sorting. Peripheral blood mononuclear cells were separated from heparinized blood by ficoll density gradient. The cells were washed in ice cold PBS containing 1% human AB serum, 5% goat serum, PE-conjugated CD3, PE-conjugated CD14, and FITC-conjugated anti-CCR5 clone 2D7 were purchased from BD Biosciences. FITC-conjugated CXCRI4 was purchased from R&D Systems. FACS® analysis was performed as described previously (34). Ligand-mediated reduction of CCR5 monoclonal antibody binding was assayed using antibodies specific to different domains of CCR5 (45523, 45531, 45549) (36). FITC-conjugated forms were purchased from R&D Systems. CCR5/HEK cells were incubated with either media, 1 μg/ml of CCL4, CCL5, or HisRS for 30 min, followed by addition of saturating amounts of FITC-conjugated monoclonal antibody.

Results

We began our studies by evaluating the ability of normal primary human leukocytes isolated from apheresis samples to migrate to either HisRS or AsnRS, known targets of autoantibodies in myositis, and SerRS, LysRS, and AspRS, which are not. In addition, we tested a recombinant NH₂-terminal deletion mutant of HisRS, HisRS-M, and a synthetic peptide (1–48 HisRS). Neither primary monocytes nor neutrophils responded chemotactically to HisRS, HisRS-M, 1–48 HisRS, AsnRS, SerRS, AspRS, or LysRS. However, percoll-isolated unfractionated lymphocytes and CD4⁺ and CD8⁺ lymphocyte subsets were chemotactored by HisRS and 1–48 HisRS. No leukocyte subset was chemotactored by HisRS-M. As can be seen in Fig. 1, the maximum chemotactic activity for HisRS was observed at 10 ng/ml (0.17 nM) for the unfractionated lymphocytes (C.I. = 2.2, P < 0.0001) and CD4⁺ lymphocytes (C.I. = 2.7, P < 0.0001). The maximal chemotactic activity was at 50 ng/ml for CD8⁺ lymphocytes. The maximum chemotactic activity for 1–48 HisRS was obtained with 100 ng/ml (18.9 nM) for unfractionated lymphocytes (C.I. = 2.3, P < 0.0001) and CD4⁺ lymphocytes (C.I. = 2.7, P < 0.0001). Again, there was a slight shift in the peak dose for CD8⁺ lymphocytes. Thus, HisRS is a more potent chemotactored than the 1–48 HisRS peptide, but both are efficacious. Unfractionated lymphocytes were not bindings are reported in the error bars. Unpaired Student t tests were performed between the media control and given chemokine concentrations. Chemotactic index (C.I.) was calculated as follows: (average number of migrating cells in sample/average number of cells in medium control). C.I. was considered significant if P < 0.001 compared with medium control. All experiments were repeated at least three times.
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chemoattracted to AsnRS (C.I. = 1.4, P > 0.05, n = 3) or SerRS (C.I. = 1.7, P ≤ 0.05, n = 3).

We investigated the effect of pertussis toxin on 1–48 HisRS-induced lymphocyte migration to determine if the response was mediated by a Goi, G-protein. Shown in Fig. 2, lymphocytes pretreated for 1 h with ≥10 ng/ml of pertussis toxin failed to migrate (68%, P < 0.0001) in response to 1–48 HisRS, suggesting that the response was largely Goi, G-protein mediated.

To clarify whether the effect of HisRS on migration was due to chemotaxis or chemokinesis, we performed cross-desensitization studies between CCL5 (RANTES) and HisRS. The ability of lymphocytes to migrate in response to CCL5 was greatly reduced, in a dose dependent manner, when the cells were preexposed to HisRS (Table I, upper panel). Conversely, the ability of lymphocytes to migrate to HisRS was greatly reduced when cells were preexposed to CCL5 (Table I, lower panel). Taken together, these data indicate that the HisRS-induced migration is chemotactic rather than chemokinetic, and furthermore that CCL5 and

Figure 1. Primary lymphocytes, CD4+ and CD8+ lymphocytes migrate to HisRS and 1–48 HisRS but neutrophils do not. Recombinant HisRS (HRS), a synthetic peptide corresponding to the first 48 amino acids of HisRS (1–48 HRS), or a deletion mutant form of HisRS that lacks the first 60 amino acids (M-HRS) were placed in the lower wells of a micro-Boydnean chamber. The concentrations of various HisRS forms are shown on the x-axis while the mean number of cells in a 200X field ± standard deviation is shown on the y-axis. Binding medium indicates background, while CXCL-12 (SDF-1) or fMLP was used as a positive control. Each condition was performed in triplicate. Each assay was performed a minimum of three times. Unpaired Student t tests were performed comparing the number of migrating cells in the binding medium vs. the number of migrating cells in individual chemoattractant concentrations. * denotes a P value ≤0.0001.

Figure 2. HisRS-induced chemotaxis is inhibited by pertussis toxin. Primary lymphocytes were incubated with increasing amounts of pertussis toxin for 1 h before being added to the upper wells of a chemotaxis chamber. Compared with binding media control, the HRS-induced migration was significantly reduced (unpaired Student t test, P < 0.0001) at 10 ng/ml. Higher concentration of pertussis toxin begins to be toxic and reduce spontaneous migration. Each condition was performed in triplicate. Each assay was performed a minimum of three times.
HisRS and CCR5 are competitive ligands for CCR5. Human IL-2–treated monocytes showed 6761 cpm of specific CCL4 binding; a 250-fold molar excess of CCL4 completely blocked all specific binding of radiolabeled CCL4. Even at a 2,000-fold weight (250-fold molar) excess, HisRS was unable to inhibit radiolabeled CCL4 binding. To explore the binding further, we radiolabeled HisRS, but unfortunately radiolabeled HisRS did not retain chemotactic activity (unpublished data). This nonchemokine ligand also did not induce calcium flux in lymphocytes, monocytes, or iDCs.

To circumvent these technical difficulties, we evaluated chimeric receptors composed of extracellular domains of CCR5 and CCR2 to determine if the receptor activation domains for CCL2 (MIP-1β) and CCL5 (RANTES) corresponded with those of HisRS (32). Earlier studies showed that extracellular domain switching between CCR2 and CCR5 could be used to map the extracellular domains needed for HIV-1 infection. We used the same chimeric receptor constructs to identify the extracellular domain(s) of CCR5 that are essential to transmit a chemotactic signal for HisRS. The first extracellular domain of CCR2 was ligated to the second, third and fourth extracellular domains of CCR5, this chimera is known as 2555. The reciprocal chimera is known as 5222. When the first two extracellular domains of CCR2 were ligated to the third and fourth extracellular domains of CCR5, the chimera was known as 2255.

As can be seen in Fig. 5, wild-type CCR5 transfected into HEK cells is chemotactically attracted to 10 ng/ml or 100 ng/ml of HisRS (C.I. = 2.6) or 1–48 HisRS (C.I. = 2.9), respectively. CCL5, a known CCR5 ligand, induced significant (P < 0.0001) migration at 10 ng/ml. As expected, the HEK cells transfected with wild-type CCR2 did not respond to HisRS, 1–48 HisRS, or CCL5, but CCL2, a known

### Table I. Cross Desensitization of Lymphocytes by HisRS and CCL5 (RANTES)

| Lower compartment (ng/ml) | Upper compartment (ng/ml) |
|--------------------------|---------------------------|
|                          | HRS 1.0       | HRS 10      | HRS 100     |
| 0                        | 50.33 ± 5.8   | 44.5 ± 7.3  | 47.17 ± 5.2 | 44.33 ± 3  |
| CCL5 1.0                 | 51.5 ± 7      | 46.33 ± 7.2 | 50.17 ± 3.1 | 50.33 ± 6  |
| CCL5 50                  | 97.67 ± 4.5   | 73.17 ± 4.8 | 62.67 ± 3.9 | 71.33 ± 5.3|
| CCL5 1000                | 83.67 ± 4.5   | 59.33 ± 2.9 | 52.5 ± 5.8  | 66.17 ± 5.4|

In the upper panel, cells were mixed with indicated concentrations of HisRS before being added to the upper wells of a chemotaxis chamber. In the lower panel, cells were mixed with the indicated concentrations of CCL5 before being added to the upper wells of a chemotaxis chamber. The mean number of cells per 200× field with a standard deviation is reported. Each condition was performed in triplicate. Each assay was performed a minimum of three times.

### Figure 3. IL-2–cultured primary monocytes migrate to HisRS. Recombinant HisRS (HRS) was placed in the lower wells of a micro-Boyden chamber. The concentrations of HRS is shown on the x-axis while the y-axis. Binding medium indicates background, while CCL4 (MIP1β) was used as a positive control. IL-2–cultured monocytes were tested for the ability of CCL4 and HRS to cross-desensitize each other. Cells were mixed with 50 ng/ml of CCL4 or 10 ng/ml of HRS before addition to the upper wells of the chemotaxis chamber. Each condition was performed in triplicate. Each assay was performed a minimum of three times.
CCR2 ligand, yielded a significant C.I. of 2.0 (P < 0.001). The 5222 construct did not transmit a HisRS, 1–48 HisRS, or CCL5-specific chemotactic signal. However, a CCL2 (C.I. = 1.8, P < 0.001) response was observed. The 2555 construct showed the opposite pattern and did not transmit a CCL2 chemotactic response but did respond to HisRS (C.I. = 1.9, P < 0.001), 1–48 HisRS (C.I. = 1.9, P < 0.001), and CCL5 (C.I. = 4.2). As noted previously, the 2255 construct was able to respond to both CCR2 and CCR5 ligands although the response was poor. Despite the demonstrably low capacity to compete for the CCL5 binding sites on CCR5, HisRS (C.I. = 2.5, P < 0.0001) and 1–48 HisRS (C.I. = 2.2, P < 0.001) induced a substantial chemotactic response in the 2255 construct expressing cells.

Epitope mapping studies indicated that a specific monoclonal antibody (clone 45531), which binds to the COOH-
terminal portion of the second extracellular loop (also known as the third extracellular domain), blocked both CCL4 and CCL5-induced chemotaxis and HIV-1 infection (36). To determine if HisRS-induced chemotaxis could also be blocked by this neutralizing antibody, we pretreated cells with either an isotype match or 45531, then placed the cells into the upper wells of a chemotaxis chamber. As can be seen in Fig. 6 A, 45531 blocked HisRS-induced chemotaxis while the isotype control did not. To demonstrate that the reverse was also true, we incubated either media, CCL5 or HisRS with CCR5/HEK for 30 min before addition of 45531 or a multidomain binding, nonneutralizing monoclonal antibody 45523. Both antibodies showed a large increase in relative fluorescence compared with the isotype control (Fig. 6 B). The binding of the multidomain binding antibody, 45523, was blocked a greater extent by CCL5 (mean 70 ± 3% SEM, n = 3)

than by HisRS (mean 45.7 ± 7.6% SEM, n = 3). However, both CCL5 (mean 54.6 ± 1.6% SEM, n = 3) and HisRS (mean 40.1 ± 8.9% SEM, n = 3) blocked 45531 binding. These studies, in combination with the binding and cross desensitization data, suggest that HisRS is a nonchemokine ligand for CCR5 that elicits a chemotactic response by interacting with, at most, the third and fourth extracellular domains of CCR5, but most likely the third extracellular domain of CCR5.

The possibility that aminoacyl–tRNA synthetases interact with receptors on antigen presenting cells with the capacity to initiate adaptive immune responses was next investigated. As immature, but not mature, DCs express CCR5 and CCR3, we determined whether recombinant HisRS, AsnRS, and SerRS were chemotactic for iDCs but not mature DCs (37–39). As can be seen in Fig. 7, iDCs demonstrated a chemotactic response to HisRS (C.I. = 1.7, P = 0.0001). In addition, the known myositis autoantigen, AsnRS, (C.I. = 1.8, P < 0.0001) induced iDCs to migrate, but SerRS did not. These data suggest that HisRS and AsnRS may be able to recruit CCR5- or CCR3-expressing iDCs to damaged muscle. 1–48 HisRS did not chemoattract iDCs, and mature DCs did not respond chemotactically to HisRS.

Immunohistochemical analysis was performed to determine if the infiltrating cells in muscle from myositis patients have corresponding chemokine receptors. Earlier studies showed that the mononuclear cells found in myositis tissue were CD4+ and CD8+ lymphocytes, iDCs and monocytes (22, 40). Several infiltrating mononuclear cells showed CCR5 staining (Fig. 8 A). Similarly, CCR3 was present in myositis infiltrates (Fig. 8 B). Some CCR5 staining was also observed in the degenerating/regenerating muscle fibers and in smooth muscle cells blood vessels of patients, but not of normal volunteers. There was no staining when...
the primary antibody was replaced with an isotype-matched control (Fig. 8 C).

As these observations suggest that CCR5-expressing leukocytes may play an important role in the pathogenesis of myositis, we investigated the in vivo expression of this receptor. As CCR5+ cells appeared to migrate into the inflamed muscle tissues in myositis patients, we investigated the possibility that the expression of CCR5 by circulating leukocytes was lower in anti-Jo-1 myositis patients (n = 7) than in unaffected individuals (n = 8). We compared the percentages of circulating CD3+CCR5+, CD14+CCR5+, CD3+CXCR4+, CD14+CXCR4+ positive cells. As expected the percent of cells positive for CD3 and either CCR5 or CXCR4 was low and not statistically different, nor did the number of CD14+CXCR4+ cells vary significantly between the two groups. There was, however, a 34% decrease in the average number of CD14+CCR5+ cells in the anti-Jo-1–positive myositis patients in comparison to the average number observed in unaffected individuals (P < 0.035, Fig. 9).

Discussion

The studies reported here have identified HisRS, and to a lesser extent an NH2-terminal peptide, 1–48 HisRS, and AsnRS as aminoacyl–tRNA synthetases having proinflammatory functions. Specifically, HisRS is a nonchemokine chemoattractant for CCR5-bearing cells that mediates its chemotactic signal by interacting with at least the third and fourth extracellular domains of the receptor, and AsnRS induces CCR3-expressing cells to migrate. Both HisRS and AsnRS chemoattract iDCs: cells known to express these receptors on their surface. SerRS chemoattracts HEK cells transfected with CCR3, but did not chemoattract iDCs. Of these three proteins, two are targeted by autoantibodies in myositis, and one, SerRS, has only been identified as an autoantigen in a few cases of systemic lupus erythematosus or rheumatoid arthritis, never in myositis. It is likely that the microenvironment of the tissue in which a molecule is exposed to the immune system determines the role that it will play in promulgating autoimmunity. Future studies will have to test whether or not an interaction between an aminoacyl–tRNA synthetase and chemokine receptors is part of the immunopathogenesis of human experimental myositis.

The potential of HisRS and AsnRS to recruit iDCs to sites of muscle inflammation and the demonstrated presence of inflammatory cells bearing CCR3, CCR5, and CD86 in myositis muscle (22) suggests that these proteins themselves

![Figure 8. Immunohistochemistry for CCR5 and CCR3. Muscle biopsy slides from patients with myositis were stained with anti–human CCR5 (A) or anti–human CCR3 (B). Arrows point to several CCR5 and CCR3-positive mononuclear cells. Isotype controls did not stain any infiltrating mononuclear cells (C). Original magnification: 340X.](image)

![Figure 9. Myositis patients have fewer CD14+CCR5+ circulating leukocytes. Peripheral blood was collected from unaffected individuals (n = 8) and anti-Jo-1–positive myositis patients (n = 7). Peripheral mononuclear cells were collected, stained for CD3, CD14, CCR5, or CXCR4 and subjected to FACS® analysis. Median values with standard deviations are plotted. The reduction observed between unaffected samples and affected samples is statistically significant as determined by paired Student t test P = 0.036.](image)
may participate in the initiation of an adaptive immune response that leads to the production of autoantibodies. It has been amply documented that antigens interacting with receptors on antigen presenting cells are processed tens of thousands of times more effectively and are therefore more immunogenic (41, 42). By engaging receptors on iDCs, these aminoacyl-tRNA synthetases may be much more likely to initiate T cell and B cell immune responses.

Studies by Blechynden and colleagues showed that inoculation of BALB/c and C57B/6 mice with naked DNA constructs for expressing HisRS led to myositis localized at the injection site (43), and there is precedent for chemotactic activities encoded in naked DNA constructs enhancing the immune response (44). Thus, HisRS and AsnRS, based on their chemotactic activity for iDCs, may initiate a cascade of immune events, beginning with antigen presentation to T lymphocytes and production of B lymphocyte stimulating cytokines that result in the production in autoantibodies in some patients. This suggests that, in the framework of the “Danger Model of Immunity” (45), autoimmunity, or at least autoantibodies, may be a consequence of a danger signal borne by the target autoantigen itself.

We report here that CCR5- and CCR3-bearing cells are present in the affected tissues. TH1 T cells, particularly CCR5+ cells, are increased at sites of inflammation in idiopathic autoimmune disease like multiple sclerosis and rheumatoid arthritis (46–48). We have shown here that this is also true in myositis. Earlier studies have similarly detected the expression of CCR5 on smooth muscle cells found in atherosclerotic plaques. Thus, a possible role for CCR5 in the amplification of autoimmune inflammation is not unprecedented. Although both genetic and environmental factors are likely to play a role in at least some cases of myositis, the details remain largely speculative (49, 50). Based on recent observations that CCR2 (51) as well as CCR5 and CCR3 expression are increased in affected tissues, the role of potential allelic variants resulting in overexpression of these chemokine receptors warrants study.

The expansion of previously selected lymphocytes in the periphery is theorized to lead to a limited TCR repertoire and increased autoreactive lymphocytes (52), but very little is known about the specific T cells supporting the production of myositis-specific autoantibodies or about the specificity of the cytotoxic T cells observed in the muscle (40, 53, 54). Indeed, very little is known about the T cells driving most autoantibody responses. The detection of TH1 cytokines in myositis tissue and the character of the immune response to HisRS (55) strongly suggest that they must participate. It is also possible that some of the aminoacyl–tRNA synthetases are autoantigens for T cells in myositis, inducing cellular immunity without inducing accompanying humoral autoantibody production.

HisRS, AsnRS, and SerRS join several other aminoacyl–tRNA synthetases that appear to have multiple physiological roles. As noted in the introduction, several type II aminoacyl-tRNA synthetases have now been demonstrated to contain somewhere in their sequence leukocyte activating functions of various types (56), and fragments of TyrRS and TrpRS have recently been reported to have cytokine-like effects on angiogenesis (57, 58). Whether these extracellular extracellular activities of aminoacyl-tRNA synthetases are the accidental by-products of evolution or have been selected for these roles remains to be discovered.

Finally, although the effects we have observed do not fully delineate the immunological events leading to the production of autoantibodies, they do extend the chain as far as iDCs, thereby potentially connecting the chemotactic activity to initiation of the adaptive immune response. These studies suggest that self antigens that possess the capacity to interact with receptors on iDC, and not just autoantigens, may play a direct role in initiation and propagation autoimmune disease.

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