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,[4] 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. Oppenheim[1]

[1]*National Cancer Institute, Center for Cancer Research, Laboratory of Molecular Immunoregulation, and* [2]*Intramural Research Support Program, SAIC Frederick, NCI-Frederick, Frederick, MD 21702*

[3]*Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892*

[4]*Department of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, MD 21218*

[5]*Institute Max von Laue, Paul Langevin, and* [6]*Department of Medicine, Michigan State University, East Lansing, MI 48823*

[7]*Department 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 NH_2-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

P.H. Plotz and J.J. Oppenheim contributed equally to this work.

Address correspondence to O.M. Zack Howard, National Cancer Institute, Center for Cancer Research, Laboratory of Molecular Immunoregulation, P.O. Box B, Frederick, MD 21702. Phone: 301-846-1348; Fax: 301-846-7042; E-mail: howardz@mail.ncifcrf.gov

**Abbreviations used in this paper:* AspRS, aspartyl–tRNA synthetase; C.I., chemotactic index; CXCR, CXC chemokine receptor; DC, dendritic cell; HisRS, histidyl–tRNA synthetase; iDC, immature dendritic cell; LysRS, lysyl–tRNA synthetase; SerRS, seryl–tRNA synthetase; TyrRS, tyrosyl–tRNA synthetase.

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 NH_2 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 repertory that is observed in human and murine autoimmune disease. Recent observations by Dohlman 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 Venrroij, 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 NH_2-terminal domain composed of amino acids 1–364 and a COOH-terminal domain composed of amino acids 365–528. The NH_2-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 chemokines, e.g., CCL3 (macrophage inflammatory protein [MIP]-1α), CCL4 (MIP-1β), CCL5 (regulated upon activation, normal T cell expressed and secreted [RANTES]), and CCL2 (monocyte chemoattractant protein [MCP]-1), are found in myositis biopsies (23–27). Thus, chemoattractants may contribute to the development or progression of idiopathic inflammatory myopathies by regulating the influx of inflammatory and immunoreactive cells into muscle tissue.

We have compared the ability of two known myositis-specific autoantigens, HisRS, and AsnRS, with three aminoacyl–tRNA synthetases not targeted in myositis, lysyl–tRNA synthetase (LysRS), aspartyl–tRNA synthetase (AspRS), and seryl–tRNA synthetase (SerRS), to act as chemoattractants for various leukocytes.

Materials and Methods

Patient Specimens. All specimens from patients were obtained under protocols approved by the Institutional Review Boards of the Clinical Center and the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Reagents. All chemokines and cytokines were obtained from the National Institutes of Health cytokine repository. Recombinant human HisRS, a deletion mutant of HisRS lacking the amino-terminal 60 amino acids (HisRS-M) and 1–48 NH_2-terminal peptide (1–48 HisRS) were prepared as reported previously (6). The decision to use this fragment was based upon the knowledge that it contains the major autoantigenic epitope and is a coiled-coil, and that granzyme B cleaves HisRS between residues 48 and 49 (unpublished data). Recombinant human AsnRS was prepared essentially as described except with an NH_2-terminal His tag (28). The sonicated bacterial extract was loaded on a TALON column (CLONTECH Laboratories, Inc.). After washing with 100 volumes of washing buffer (20 mM Tris-HCl, 1 M NaCl, 5 mM $MgCl_2$, EDTA free protease inhibitor tablets), the synthetase was eluted with a buffer containing 20 mM Tris-HCl, 100 mM NaCl, 150 mM imidazole, and 10% glycerol and imidazole removed on PD10 columns (Amersham Biotech). The protein was then concentrated to 5 mg/ml in HEPES buffer, pH 7.2, 100 mM NaCl, 5 mM $MgCl_2$, and 10% glycerol.

Human SerRS was expressed and purified as described previously and dialyzed against 20 mM HEPES buffer, pH 7.2, 100 mM NaCl, 5 mM $MgCl_2$ and 10% glycerol (29). Recombinant human LysRS (his-tagged) and AspRS (biotin-ubiquitin tagged) were expressed in bacteria and purified by affinity chromatography (unpublished data). All other reagents were purchased from Sigma-Aldrich unless otherwise noted.

Cells. Primary human leukocytes were isolated from fresh normal donor apheresis packs under an approved human subjects protocol as reported previously (30). In some studies, percoll purified lymphocytes or monocytes were cultured at 10^6 cells/ml in RPMI-1640 (Bio Whittaker) containing 10% fetal bovine serum (HyClone) and 2 mM glutamine, 100 U/ml penicillin, and streptomycin (Quality Biologicals) with 100 U/ml of rhuIL-2 for 3 d in a 5% CO_2 humidified tissue culture incubator. Lymphocyte subsets were isolated using lymphocyte subset purification columns as directed by the manufacturer (R&D Systems). HEK-293 cells were cultured in Dulbecco's modified Eagle's medium (Bio Whittaker) containing 10% fetal bovine serum (HyClone) and 2 mM glutamine, 100 U/ml penicillin, and streptomycin (Quality

Biologicals). Parental HEK-293 cells were transfected with linearized wild-type CCR5, CCR1, CCR3, or variant mammalian expression constructs by electroporation (31). Linearized chimeric receptor constructs (32) were electroporated along with linearized pcDNA3 (Invitrogen) in a 10:1 ratio. After selection in media containing 800 μg/ml Geneticin (Life Technologies) for 2 wk, cells were used for analysis.

Immature dendritic cells (iDCs) were generated from purified human peripheral blood monocytes (>95%) which were incubated at 10^6 cells/ml in G4 medium (RPMI 1640, 10% FBS, 2 mM glutamine, 25 mM HEPES, 100 U/ml penicillin, 100 mg/ml streptomycin, 50 ng/ml of rhGM-CSF, and 50 ng/ml of rhIL-4) at 37°C in a humidified CO_2 (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 $CD1a^+$, $CD14^-$, $CD40^{low}$, $CD83^-$, $CD86^{low}$, HLA-DR^{medium}. Mature dendritic cells (DCs) were generated by treating the iDCs for 48 h with 50 ng/ml of rhTNF-α.

Chemotaxis. Cells were resuspended in binding media (RPMI 1640 containing 1% bovine serum albumin, 25 mM HEPES, pH 8.0) at 1–5 × 10^6 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 chemoattractants. 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 CO_2 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.) were 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 ^{125}I 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 × 10^6 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 acetone 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 CXCR4 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 NH_2-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 chemoattracted by HisRS and 1–48 HisRS. No leukocyte subset was chemoattracted 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, $P < 0.001$). 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 chemoattractant than the 1–48 HisRS peptide, but both are efficacious. Unfractionated lymphocytes were not

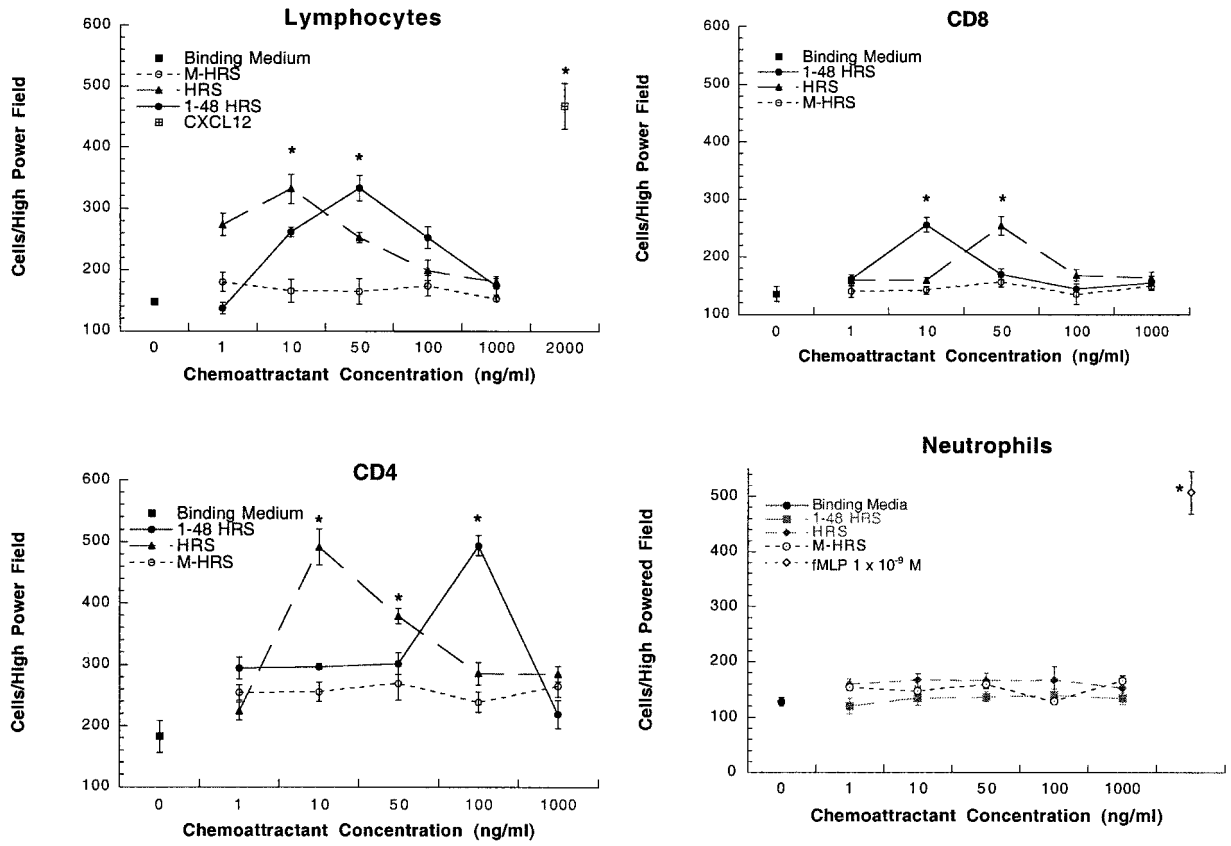

Figure 1. Primary lymphocytes, $CD4^+$ and $CD8^+$ lymphocytes migrate to HisRS and 1–48 HRS but neutrophils do not. Recombinant HisRS (HRS), a synthetic peptide corresponding to the first 48 amino acids of HisRS (1–48 HRS), or a deletion mutant form of HisRS that lacks the first 60 amino acids (M-HRS) were placed in the lower wells of a micro-Boyden chamber. The concentrations of various HisRS forms are shown on the x-axis while the mean number of cells in a 200× 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.

chemoattracted to AsnRS (C.I. = 1.4, $P > 0.05$, $n = 3$) or SerRS (C.I. = 1.7, $P \leq 0.05$, $n = 3$).

We investigated the effect of pertussis toxin on 1–48 HisRS-induced lymphocyte migration to determine if the response was mediated by a $G\alpha_i$ 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 $G\alpha_i$ 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

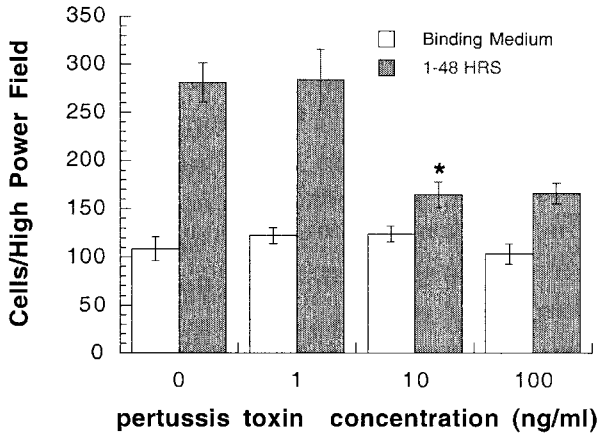

Figure 2. HisRS-induced chemotaxis is inhibited by pertussis toxin. Primary lymphocytes were incubated with increasing amounts of pertussis toxin for 1 h before being added to the upper wells of a chemotaxis chamber. Compared with binding media control, the HRS-induced migration was significantly reduced (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.

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

Lower compartment (ng/ml)	Upper compartment (ng/ml)			
	0	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
	0	CCL5 1.0	CCL5 50	CCL5 1000
0	66.5 ± 7.4	60.5 ± 2.2	59.17 ± 3.1	56.67 ± 2.6
HRS 1.0	71.67 ± 8.7	68 ± 7.1	58.33 ± 1.6	55.17 ± 2.04
HRS 10	128.5 ± 14.1	111 ± 15.7	61.83 ± 6.1	55.5 ± 1.5
HRS 100	81.67 ± 6.3	77.5 ± 6.3	67.67 ± 5.3	56.5 ± 3.3

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.

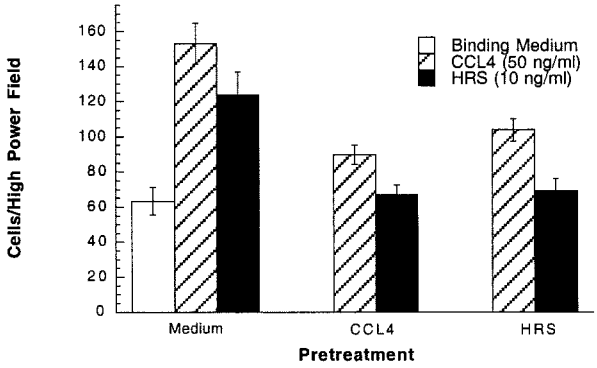

Figure 3. IL-2–cultured primary monocytes migrate to HisRS. Recombinant HisRS (HRS) was placed in the lower wells of a micro-Boyden chamber. The concentrations of HRS is shown on the x-axis while the mean number of cells in a 200× field ± standard deviation is shown on the y-axis. Binding medium indicates 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.

HisRS share a common receptor. As CCL5 interacts with CCR1, CCR3, and CCR5, this suggested that HisRS might be acting on one or more of these receptors.

IL-2 treatment has been reported to enhance the expression of chemokine receptors by monocytes (35). Monocytes treated for 3 d with 100 U/ml of human IL-2 did migrate in response to HisRS (10 ng/ml). Pretreatment of such IL-2–stimulated monocytes with HisRS or CCL4 (MIP-1β), which uses predominantly CCR5, also inhibited HisRS-induced chemotaxis (Fig. 3).

To identify the targeted receptor, we assayed the chemotactic responses of a number of single chemokine receptor transfected HEK-293 cells. Neither CCR1 nor CCR3 transfected cells responded to HisRS or 1–48 HisRS (data shown in Fig. 4 A). However, CCR5/HEK transfectants did respond to HisRS in a dose-dependent manner (Fig. 4 A). As observed for unfractionated and $CD4^+$ lymphocytes, the maximum response was at 10 ng/ml (C.I. = 4.8, $P < 0.0001$). Additionally, HOS/CD4/CCR5 transfectants also migrated to HisRS (unpublished data). As we already suspected that another CC receptor that was less highly expressed on lymphocytes might be a receptor for SerRS or AsnRS, we also tested them on a number of CC chemokine transfected HEK cells. Both SerRS and AsnRS induced CCR3-transfected cells to migrate in a dose-dependent manner. The maximum response for each was at 100 ng/ml (C.I. = 2.0, $P < 0.0001$; Fig. 4 B).

The desensitizing effects of HisRS on CCL4 signal transduction led us to determine whether HisRS and CCL4 are competitive ligands for CCR5. Human IL-2–treated monocytes showed 6761 cpm of specific CCL4 binding; a 250-fold molar excess of cold 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 (MCP1) 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 chemoattracted 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

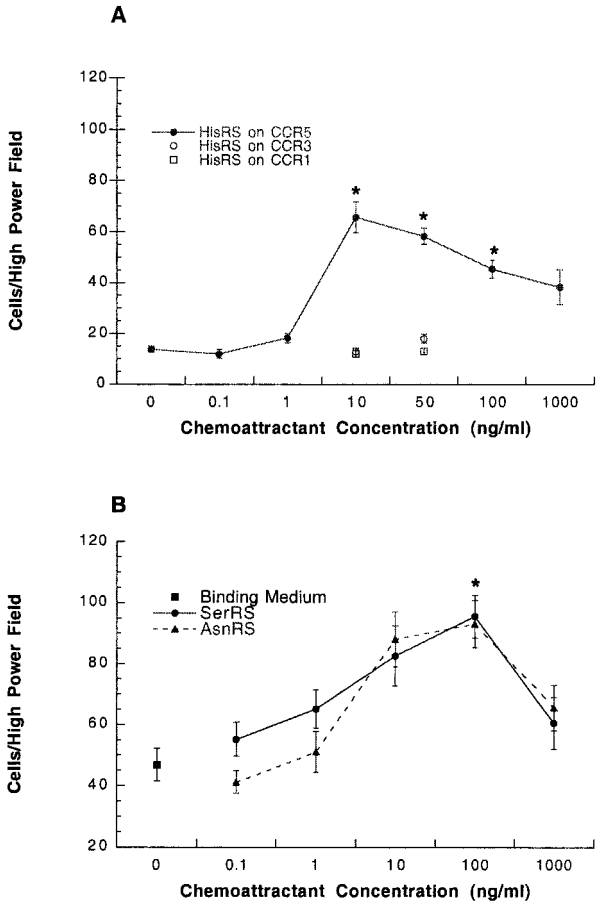

Figure 4. (A) CCR5 transfected HEK/293 cells (CCR5/HEK) migrate to HisRS. (B) CCR3 transfected HEK/293 cells (CCR3/HEK) migrate to AsnRS and SerRS. Recombinant HisRS, SerRS, or AsnRS was placed in the lower wells of a micro-Boyden chamber. The open circles (CCR3) and open squares (CCR1) show the lack of chemotaxis of CCR3 and CCR1 transfected HEK cells to HisRS. The concentrations of are shown on the x-axis while the mean number of cells in a 200× filed ± standard deviation is shown on the y-axis. 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 media vs. the number of migrating cells in individual chemoattractant concentrations. * denotes a P value ≤0.0001.

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-

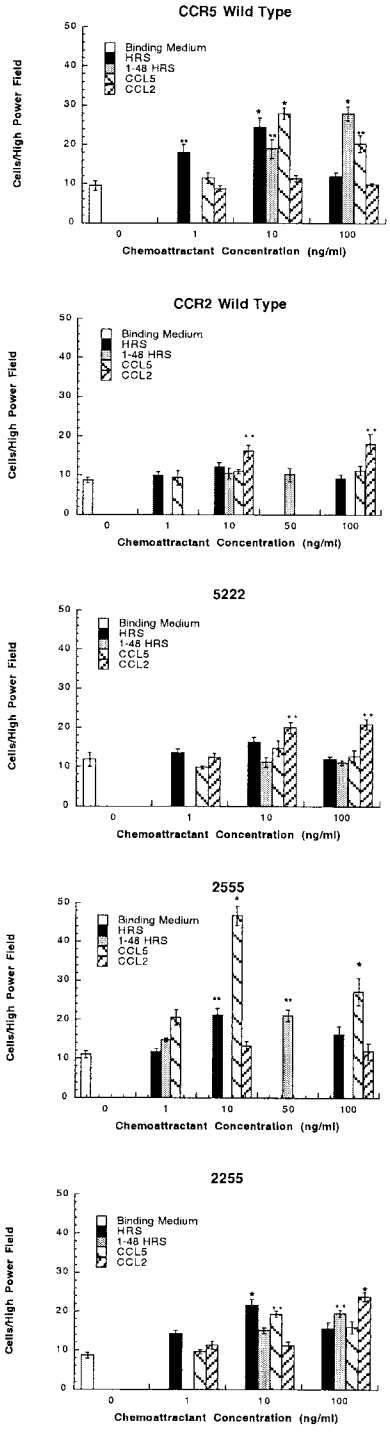

Figure 5. Migration of CCR5 and CCR2 chimeras to HisRS or 1–48 HRS. CCR5, CCR2, and chimeric constructs were expressed in HEK/293 cells. Recombinant HisRS (HRS) or the 1–48 HRS peptide was placed in the lower wells of a micro-Boyden chamber. The concentrations of HRS is shown on the x-axis while the mean number of cells in a 200× field ± standard deviation is shown on the y-axis. 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 media vs. the number of migrating cells in individual chemoattractant concentrations. * denotes a P value ≤0.0001, ** denotes a P value of ≤0.001.

terminal portion of the second extracellular loop (also know 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.

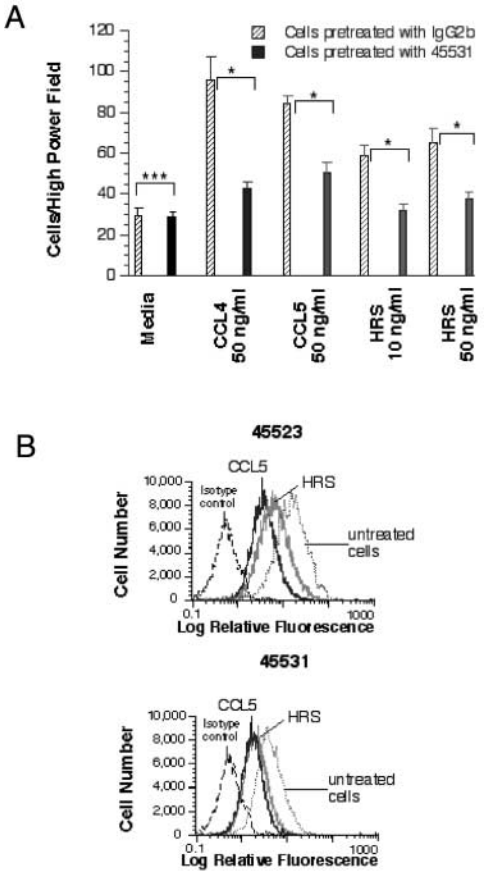

Figure 6. (A) CCR5 neutralizing antibody blocks CCL4, CCL5, and HisRS-induced CCR5/HEK migration. (B) CCL5 and HisRS (HRS) block domain specific anti-CCR5 binding. CCR5/HEK cells were pretreated with either isotype control antibody, shown in hatched bars, or neutralizing antibody 45531, shown in solid bars, then subjected to chemotaxis. Pretreatment with 45531 significantly inhibited all induced chemotaxis (* indicates $P < 0.0001$ between sets designated by a bracket), but did not inhibit spontaneous migration (*** indicates $P > 0.2$). A representative FACS® experiment of three is shown. Pretreatment with CCL5 blocked multidomain specific monoclonal antibody 45523 binding to a greater extend than did HisRS (HRS). However, both CCL5 and HisRS blocked extracellular loop 2 specific antibody 45531 binding, equally.

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 response 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

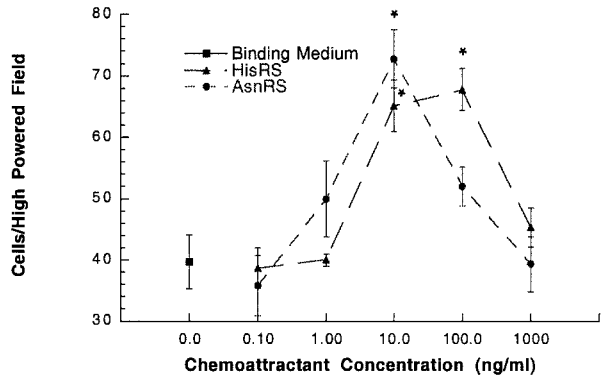

Figure 7. iDCs are chemoattracted to HisRS and AsnRS. Immature DCs were generated from peripheral monocytes as described in Materials and Methods. Recombinant HisRS (HRS) or recombinant AsnRS (NRS) was placed in the lower wells of a micro-Boyden chamber. The concentrations are shown on the x-axis while the mean number of cells in a 200× field ± standard deviation is shown on the y-axis. 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 media vs. the number of migrating cells in individual chemoattractant concentrations. * denotes a P value ≤0.0001.

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).

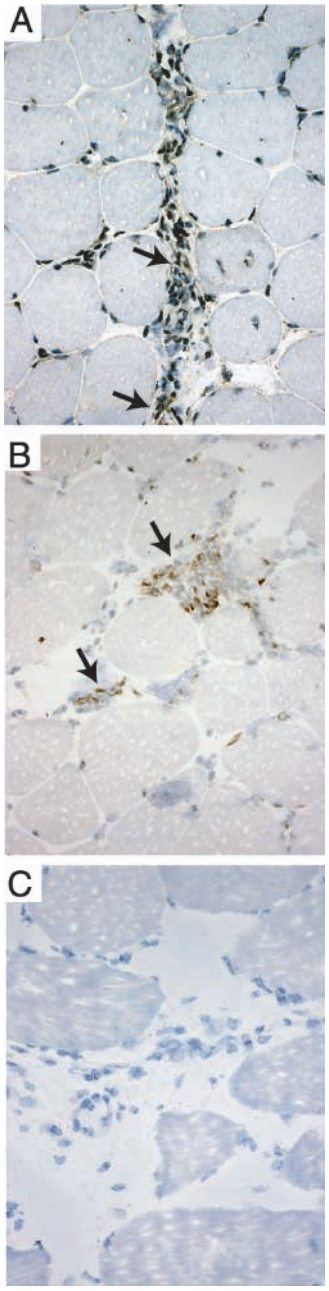

Figure 8. Immunohistochemistry for CCR5 and CCR3. Muscle biopsy slides from patients with myositis were stained with anti–human CCR5 (A) or anti–human CCR3 (B). Arrows point to several CCR5 and CCR3-positive mononuclear cells. Isotype controls did not stain any infiltrating mononuclear cells (C). Original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

Discussion

The studies reported here have identified HisRS, and to a lesser extent an NH_2-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

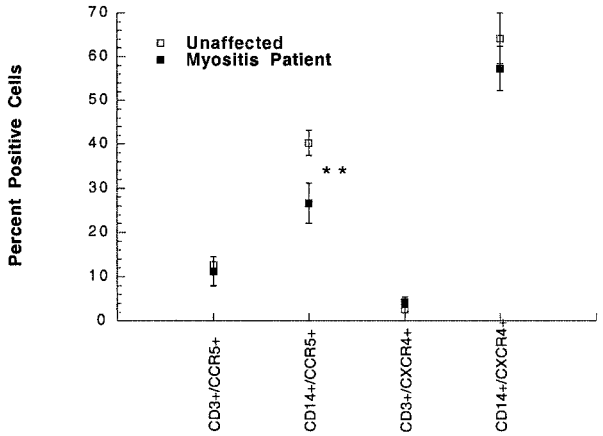

Figure 9. Myositis patients have fewer $CD14^+CCR5^+$ circulating leukocytes. Peripheral blood was collected from unaffected individuals ($n = 8$) and anti-Jo-1–positive myositis patients ($n = 7$). Peripheral mononuclear cells were collected, stained for CD3, CD14, CCR5, or CXCR4 and subjected to FACS® 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 $P = 0.036$.

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 extracurricular 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 autoantibodies, may play a direct role in initiation and promulgation autoimmune disease.

Dr. Israel F. Charo, icharo@gladstone.ucsf.edu, kindly provided the CCR5/CCR2 chimeric receptor constructs. Dr. Phillip Murphy of the National Institute of Allergy and Infectious Diseases kindly helped us to carry out calcium flux studies. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the U.S. Government.

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, under contract no. N01-CO-12400 and from the Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Submitted: 4 February 2002
Revised: 10 June 2002
Accepted: 6 August 2002

References

1. Targoff, I.N. 2000. Update on myositis-specific and myositis-associated autoantibodies. *Curr. Opin. Rheumatol.* 12:475–481.
2. Hengstman, G.J., B.G. van Engelen, W.T. Vree Egberts, and W.J. van Venrooij. 2001. Myositis-specific autoantibodies: overview and recent developments. *Curr. Opin. Rheumatol.* 13:476–482.
3. Bolgarin, R.N., N.V. Rodnin, and L.L. Sidorik. 1998. Autoantibodies to tryptophanyl-tRNA synthetase in systemic autoimmune diseases. *Mol. Biol. (Mosk).* 32:745–749.
4. Ramsden, D.A., J. Chen, F.W. Miller, V. Misener, R.M. Bernstein, K.A. Siminovitch, and F.W. Tsui. 1989. Epitope mapping of the cloned human autoantigen, histidyl-tRNA synthetase. Analysis of the myositis-associated anti-Jo-1 autoimmune response. *J. Immunol.* 143:2267–2272.
5. Freist, W., J.F. Verhey, A. Ruhlmann, D.H. Gauss, and J.G. Arnez. 1999. Histidyl-tRNA synthetase. *Biol. Chem.* 380: 623–646.
6. Raben, N., R. Nichols, J. Dohlman, P. McPhie, V. Sridhar, C. Hyde, R. Leff, and P. Plotz. 1994. A motif in human histidyl-tRNA synthetase which is shared among several aminoacyl-tRNA synthetases is a coiled-coil that is essential for enzymatic activity and contains the major autoantigenic epitope. *J. Biol. Chem.* 269:24277–24283.
7. Maldonado, M.A., V. Kakkanaiah, G.C. MacDonald, F. Chen, E.A. Reap, E. Balish, W.R. Farkas, J.C. Jennette, M.P. Madaio, B.L. Kotzin, et al. 1999. The role of environ-

mental antigens in the spontaneous development of autoimmunity in MRL-lpr mice. *J. Immunol.* 162:6322–6330.
8. Nagaraju, K., N. Raben, L. Loeffler, T. Parker, P.J. Rochon, E. Lee, C. Danning, R. Wada, C. Thompson, G. Bahtiyar, et al. 2000. Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. *Proc. Natl. Acad. Sci. USA.* 97:9209–9214.
9. Brendel, V., J. Dohlman, B.E. Blaisdell, and S. Karlin. 1991. Very long charge runs in systemic lupus erythematosus-associated autoantigens. *Proc. Natl. Acad. Sci. USA.* 88:1536–1540.
10. Dohlman, J.G., A. Lupas, and M. Carson. 1993. Long charge-rich alpha-helices in systemic autoantigens. *Biochem. Biophys. Res. Commun.* 195:686–696.
11. Casciola-Rosen, L., A. Rosen, M. Petri, and M. Schlissel. 1996. Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: implications for coagulation events and antigenic spread in systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA.* 93:1624–1629.
12. Casciola-Rosen, L., and A. Rosen. 1997. Ultraviolet light-induced keratinocyte apoptosis: a potential mechanism for the induction of skin lesions and autoantibody production in LE. *Lupus.* 6:175–180.
13. Rosen, A., and L. Casciola-Rosen. 1999. Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. *Cell Death Differ.* 6:6–12.
14. Andrade, F., S. Roy, D. Nicholson, N. Thornberry, A. Rosen, and L. Casciola-Rosen. 1998. Granzyme B directly and efficiently cleaves several downstream caspase substrates: implications for CTL-induced apoptosis. *Immunity.* 8:451–460.
15. Tan, E.M. 1996. Autoantibodies–what do they recognize? *Verh. Dtsch. Ges. Pathol.* 80:1–11.
16. Utz, P.J., and P. Anderson. 1998. Posttranslational protein modifications, apoptosis, and the bypass of tolerance to autoantigens. *Arthritis Rheum.* 41:1152–1160.
17. Utz, P.J., M. Hottelet, W.J. van Venrooij, and P. Anderson. 1998. Association of phosphorylated serine/arginine (SR) splicing factors with the U1-small ribonucleoprotein (snRNP) autoantigen complex accompanies apoptotic cell death. *J. Exp. Med.* 187:547–560.
18. Doyle, H.A., and M.J. Mamula. 2001. Post-translational protein modifications in antigen recognition and autoimmunity. *Trends Immunol.* 22:443–449.
19. Wakasugi, K., and P. Schimmel. 1999. Highly differentiated motifs responsible for two cytokine activities of a split human tRNA synthetase. *J. Biol. Chem.* 274:23155–23159.
20. Wakasugi, K., and P. Schimmel. 1999. Two distinct cytokines released from a human aminoacyl-tRNA synthetase. *Science.* 284:147–151.
21. Martinis, S.A., P. Plateau, J. Cavarelli, and C. Florentz. 1999. Aminoacyl-tRNA synthetases: a new image for a classical family. *Biochimie.* 81:683–700.
22. Nagaraju, K., N. Raben, M.L. Villalba, C. Danning, L.A. Loeffler, E. Lee, N. Tresser, A. Abati, P. Fetsch, and P.H. Plotz. 1999. Costimulatory markers in muscle of patients with idiopathic inflammatory myopathies and in cultured muscle cells. *Clin. Immunol.* 92:161–169.
23. Adams, E.M., J. Kirkley, G. Eidelman, J. Dohlman, and P.H. Plotz. 1997. The predominance of beta (CC) chemokine transcripts in idiopathic inflammatory muscle diseases. *Proc. Assoc. Am. Physicians.* 109:275–285.
24. Confalonieri, P., P. Bernasconi, P. Megna, S. Galbiati, F. Cornelio, and R. Mantegazza. 2000. Increased expression of beta-chemokines in muscle of patients with inflammatory myopathies. *J. Neuropathol. Exp. Neurol.* 59:164–169.
25. De Rossi, M., P. Bernasconi, F. Baggi, M.R. de Waal, and R. Mantegazza. 2000. Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation. *Int. Immunol.* 12: 1329–1335.
26. Liprandi, A., C. Bartoli, D. Figarella-Branger, J.F. Pellissier, and H. Lepidi. 1999. Local expression of monocyte chemoattractant protein-1 (MCP-1) in idiopathic inflammatory myopathies. *Acta Neuropathol. (Berl.).* 97:642–648.
27. Lundberg, I.E. 2000. The role of cytokines, chemokines, and adhesion molecules in the pathogenesis of idiopathic inflammatory myopathies. *Curr. Rheumatol. Rep.* 2:216–224.
28. Beaulande, M., N. Tarbouriech, and M. Hartlein. 1998. Human cytosolic asparaginyl-tRNAsynthetase: cDNA sequence, functional expression in Escherichia coli and characterization as human autoantigen. *Nucleic Acids Res.* 26:521–524.
29. Vincent, C., N. Tarbouriech, and M. Hartlein. 1997. Genomic organization, cDNA sequence, bacterial expression and purification of human seryl-tRNA synthase. *Eur. J. Biochem.* 250:77–84.
30. Grimm, M.C., A. Ben Baruch, D.D. Taub, O.M. Howard, J.H. Resau, J.M. Wang, H. Ali, R. Richardson, R. Snyderman, and J.J. Oppenheim. 1998. Opiates transdeactivate chemokine receptors: delta and mu opiate receptor-mediated heterologous desensitization. *J. Exp. Med.* 188:317–325.
31. Howard, O.M., T. Korte, N.I. Tarasova, M. Grimm, J.A. Turpin, W.G. Rice, C.J. Michejda, R. Blumenthal, and J.J. Oppenheim. 1998. Small molecule inhibitor of HIV-1 cell fusion blocks chemokine receptor-mediated function. *J. Leukoc. Biol.* 64:6–13.
32. Atchison, R.E., J. Gosling, F.S. Monteclaro, C. Franci, L. Digilio, I.F. Charo, and M.A. Goldsmith. 1996. Multiple extracellular elements of CCR5 and HIV-1 entry: dissociation from response to chemokines. *Science.* 274:1924–1926.
33. Yang, D., O.M. Howard, Q. Chen, and J.J. Oppenheim. 1999. Cutting edge: immature dendritic cells generated from monocytes in the presence of TGF-beta 1 express functional C-C chemokine receptor 6. *J. Immunol.* 163:1737–1741.
34. Howard, O.M., R.A. Kirken, G.G. Garcia, R.H. Hackett, and W.L. Farrar. 1995. Structural domains of interleukin-2 receptor beta critical for signal transduction: kinase association and nuclear complex-formation. *Biochem. J.* 306(Pt 1): 217–224.
35. Moriuchi, H., M. Moriuchi, and A.S. Fauci. 1997. Cloning and analysis of the promoter region of CCR5, a coreceptor for HIV-1 entry. *J. Immunol.* 159:5441–5449.
36. Lee, B., M. Sharron, C. Blanpain, B.J. Doranz, J. Vakili, P. Setoh, E. Berg, G. Liu, H.R. Guy, S.R. Durell, et al. 1999. Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and corecptor function. *J. Biol. Chem.* 274:9617–9626.
37. Granelli-Piperno, A., B. Moser, M. Pope, D. Chen, Y. Wei, F. Isdell, U. O'Doherty, W. Paxton, R. Koup, S. Mojsov, et al. 1996. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. *J. Exp. Med.* 184:2433–2438.
38. Sozzani, S., W. Luini, A. Borsatti, N. Polentarutti, D. Zhou,

L. Piemonti, G. D'Amico, C.A. Power, T.N. Wells, M. Gobbi, et al. 1997. Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. *J. Immunol.* 159:1993–2000.
39. Delgado, E., V. Finkel, M. Baggiolini, C.R. Mackay, R.M. Steinman, and A. Granelli-Piperno. 1998. Mature dendritic cells respond to SDF-1, but not to several beta- chemokines. *Immunobiology.* 198:490–500.
40. Emslie-Smith, A.M., K. Arahata, and A.G. Engel. 1989. Major histocompatibility complex class I antigen expression, immunolocalization of interferon subtypes, and T cell-mediated cytotoxicity in myopathies. *Hum. Pathol.* 20:224–231.
41. Ronchetti, A., P. Rovere, G. Iezzi, G. Galati, S. Heltai, M.P. Protti, M.P. Garancini, A.A. Manfredi, C. Rugarli, and M. Bellone. 1999. Immunogenicity of apoptotic cells in vivo: role of antigen load, antigen-presenting cells, and cytokines. *J. Immunol.* 163:130–136.
42. Henry, F., O. Boisteau, L. Bretaudeau, B. Lieubeau, K. Meflah, and M. Gregoire. 1999. Antigen-presenting cells that phagocytose apoptotic tumor-derived cells are potent tumor vaccines. *Cancer Res.* 59:3329–3332.
43. Blechynden, L.M., M.A. Lawson, H. Tabarias, M.J. Garlepp, J. Sherman, N. Raben, and C.M. Lawson. 1997. Myositis induced by naked DNA immunization with the gene for histidyl-tRNA synthetase. *Hum. Gene Ther.* 8:1469–1480.
44. Biragyn, A., K. Tani, M.C. Grimm, S. Weeks, and L.W. Kwak. 1999. Genetic fusion of chemokines to a self tumor antigen induces protective, T-cell dependent antitumor immunity. *Nat. Biotechnol.* 17:253–258.
45. Gallucci, S., and P. Matzinger. 2001. Danger signals: SOS to the immune system. *Curr. Opin. Immunol.* 13:114–119.
46. Kim, C.H., and H.E. Broxmeyer. 1999. Chemokines: signal lamps for trafficking of T and B cells for development and effector function. *J. Leukoc. Biol.* 65:6–15.
47. Qin, S., J.B. Rottman, P. Myers, N. Kassam, M. Weinblatt, M. Loetscher, A.E. Koch, B. Moser, and C.R. Mackay. 1998. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J. Clin. Invest.* 101:746–754.
48. Patel, D.D., J.P. Zachariah, and L.P. Whichard. 2001. CXCR3 and CCR5 ligands in rheumatoid arthritis synovium. *Clin. Immunol.* 98:39–45.
49. Shamim, E.A., L.G. Rider, and F.W. Miller. 2000. Update on the genetics of the idiopathic inflammatory myopathies. *Curr. Opin. Rheumatol.* 12:482–491.
50. Leff, R.L., L.A. Love, F.W. Miller, S.J. Greenberg, E.A. Klein, M.C. Dalakas, and P.H. Plotz. 1992. Viruses in idiopathic inflammatory myopathies: absence of candidate viral genomes in muscle. *Lancet.* 339:1192–1195.
51. Bartoli, C., M. Civatte, J.F. Pellissier, and D. Figarella-Branger. 2001. CCR2A and CCR2B, the two isoforms of the monocyte chemoattractant protein-1 receptor are up-regulated and expressed by different cell subsets in idiopathic inflammatory myopathies. *Acta Neuropathol. (Berl.).* 102:385–392.
52. Mackall, C.L., C.V. Bare, L.A. Granger, S.O. Sharrow, J.A. Titus, and R.E. Gress. 1996. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. *J. Immunol.* 156:4609–4616.
53. Hohlfeld, R., A.G. Engel, N. Goebels, and L. Behrens. 1997. Cellular immune mechanisms in inflammatory myopathies. *Curr. Opin. Rheumatol.* 9:520–526.
54. Nagaraju, K. 2001. Update on immunopathogenesis in inflammatory myopathies. *Curr. Opin. Rheumatol.* 13:461–468.
55. Miller, F.W., K.A. Waite, T. Biswas, and P.H. Plotz. 1990. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. *Proc. Natl. Acad. Sci. USA.* 87:9933–9937.
56. Martinis, S.A., P. Plateau, J. Cavarelli, and C. Florentz. 1999. Aminoacyl-tRNA synthetases: a family of expanding functions. Mittelwihr, France, October 10-15, 1999. *EMBO J.* 18:4591–4596.
57. Wakasugi, K., B.M. Slike, J. Hood, A. Otani, K.L. Ewalt, M. Friedlander, D.A. Cheresh, and P. Schimmel. 2002. A human aminoacyl-tRNA synthetase as a regulator of angiogenesis. *Proc. Natl. Acad. Sci. USA.* 99:173–177.
58. Otani, A., B.M. Slike, M.I. Dorrell, J. Hood, K. Kinder, K.L. Ewalt, D. Cheresh, P. Schimmel, and M. Friedlander. 2002. A fragment of human TrpRS as a potent antagonist of ocular angiogenesis. *Proc. Natl. Acad. Sci. USA.* 99:178–183.