Hsp60 in Inflamed Muscle Tissue Is the Target of Regulatory Autoreactive T Cells in Patients With Juvenile Dermatomyositis

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Objective. Juvenile dermatomyositis (DM) is an autoimmune disease of unknown origin characterized by muscle weakness and skin manifestations. No definite autoantigen has yet been identified. Heat-shock proteins (HSPs) can be up-regulated at sites of inflammation, and immune reactivity to Hsp60 is suggested to play a regulatory role in various chronic inflammatory diseases. The purpose of this study was to determine whether Hsp60 could serve as an autoantigen in juvenile DM.

Methods. Muscle tissue from 4 patients with juvenile DM and 1 healthy control subject without evidence of muscle disease was stained for Hsp60. Peripheral blood mononuclear cells (PBMCs) from 22 patients and 10 healthy control subjects were tested for T cell proliferation induced by human and microbial Hsp60. Cytokine production in response to Hsp60 was examined in 15 patients and 6 healthy controls. T cell reactivity to Hsp60 was determined in muscle biopsy samples from 2 patients.

Results. We found significantly increased T cell proliferation to human Hsp60 in PBMCs from juvenile DM patients, which was higher during disease remission. Following in vitro activation with Hsp60, significant amounts of tumor necrosis factor α, interleukin-1β (IL-1β), and IL-10 were produced. In contrast to muscle biopsy samples from healthy controls, samples from juvenile DM patients showed up-regulation of Hsp60, induction of T cell proliferation, and production of cytokines. Production of proinflammatory cytokines by muscle-derived cells in response to Hsp60 was associated with a poor clinical prognosis, whereas human Hsp60–specific induction of IL-10 was followed by clinical remission.

Conclusion. These findings suggest that human (self) Hsp60 is a disease-relevant autoantigen in juvenile DM. The difference in T cell response with regard to disease activity indicates an immune regulatory effect of Hsp60-specific T cells, opening up perspectives for antigen-specific immunotherapy.

Juvenile dermatomyositis (DM) is an inflammatory myopathy of unknown origin in which the immune system targets the microvasculature of the skeletal muscles, the skin, and, to a lesser extent, other organs. This leads to the characteristic clinical picture of proximal muscle weakness and a typical rash, particularly affecting the face and hands (1). Current treatment of juvenile DM consists of immunosuppressive agents, which dampen all immune responses, pathogenic as well as protective, and can cause serious side effects (2).

The pathogenesis of juvenile DM is not fully understood. As in most human autoimmune diseases, it is thought that T cells elicit proinflammatory immune reactions against self antigens that initiate the disease (3,4). Possibly as a consequence of the autoreactive T cell insult, B cells, autoantibodies, and complement...
activation clearly play a role in the immune pathogenesis of juvenile DM (1). Once the disease progresses, the inflammatory immune reaction broadens, since an immune response to a single autoantigen can trigger immune responses to neighboring autoantigens. This process ultimately leads to a large repertoire of immune responses to multiple self antigens. At this stage, it is unlikely that the disease-initiating autoantigens still play a decisive role in the perpetuation of the inflammation.

Identification of self antigens that play an immunomodulatory role throughout the disease process may be important for diagnostic purposes. In addition, such self antigens may ultimately serve as targets for antigen-specific immunotherapy (5). However, despite much effort by many groups of investigators, disease-triggering or disease-regulating autoantigens have not been identified in juvenile DM.

Over the last decade, it has been recognized that T cells responding to heat-shock proteins (HSPs) are part of a normal immunoregulatory response that has the potential to dominantly control proinflammatory responses and chronic inflammatory disease (6). HSPs are present in the cells of all living organisms, are highly conserved during evolution, and play a role in various cellular processes. Under physiologic conditions, some of these proteins function as molecular chaperones. During cellular stress, as in inflammation, their expression is up-regulated, and they serve both as a danger signal that can start an inflammatory reaction and as an inducer of regulatory T cell responses that can dampen inflammation (7,8). It has been shown both in animal models and in humans with diseases such as arthritis, type 1 diabetes mellitus, and atherosclerosis that self HSPs that are produced and released during cellular stress can act as antigenic regulators of the inflammatory response (9–15).

In muscle tissue, an increased expression of HSPs is found in response to stress, such as muscle damage and exercise-induced stress (16–18). Interestingly, an increase in HSPs in cardiac muscle biopsy tissues obtained after anoxemia and following cardiac surgery is associated with myocardial protection (19). However, to date, little is known about the role of Hsp60 in inflammatory muscle disorders. In muscle tissue from patients with inflammatory myositis, an increased expression of Hsp60 has been reported (20). Hsp60 gene expression was also reported to be elevated in muscle samples from patients with untreated juvenile DM (21).

We hypothesized that up-regulated Hsp60 in inflamed muscle tissue in patients with inflammatory myopathies could also serve as an autoantigenic target for self-reactive T cells. Thus, we investigated the expression of Hsp60 in inflamed muscle tissue from patients with juvenile DM as well as the in vitro reactivity of peripheral blood mononuclear cells (PBMCs) from patients with juvenile DM against human and microbial Hsp60. Finally, we examined in vitro T cell responses to self Hsp60 in muscle biopsy samples from patients with juvenile DM.

**PATIENTS AND METHODS**

**Patients and controls.** Blood was obtained from 22 juvenile DM patients (13 girls and 9 boys; mean ± SEM age 9.5 ± 3.4 years) who fulfilled the diagnostic criteria of Bohan and Peter (22). Characteristics of the 22 study patients are shown in Table 1. Clinical evidence of disease activity was considered to be present when there were typical cutaneous alterations (Gottron’s papules, heliotrope rash, and nailfold...
hyperemia) and muscle weakness. Muscle weakness was assessed with the Childhood Myositis Assessment Scale (CMAS), which is specifically designed to determine the functional consequences of proximal muscle strength and endurance (23). The CMAS has a potential range of 0–53, with higher scores indicating greater muscle strength and endurance. Blood samples were used to measure markers of generalized inflammation (such as the erythrocyte sedimentation rate), as well as more specific markers of muscle inflammation (such as the muscle enzymes creatine kinase, aldolase, and the transaminases). Tests for aninuclear antibodies were performed in 14 of the 22 patients and the findings were positive in 2 children with active disease (titer of 1:4,000). Myositis-specific and myositis-associated antibodies were negative in the majority of the patients (data not shown).

Skin manifestations of juvenile DM and/or a decrease in the CMAS score together with an increase in the blood marker levels defined the presence of active disease (24). Absence of these parameters defined disease remission. Of the 22 children with juvenile DM, 10 were in clinical and biochemical remission and 12 had active disease; samples from 6 of the 12 with active disease were obtained at the time of diagnosis.

As controls, we obtained peripheral blood samples from 10 healthy children (5 male and 5 female; mean ± SEM age 8.3 ± 2.8 years) who were undergoing minor surgical procedures. For analysis of cytokine production, blood samples from 15 of the 22 patients (8 girls and 7 boys; mean ± SEM age 10.1 ± 2.9 years) were used. PBMCs from 6 healthy children (mean ± SEM age 7.4 ± 5.2 years) were used as controls.

Sections of muscle tissue from 4 patients with juvenile DM and 1 healthy control subject without muscle disease were stained for Hsp60. The muscle tissue was obtained by magnetic resonance imaging–directed needle biopsy at the site of the musculus vastus lateralis. Muscle tissue from 2 other patients (patients 1 and 2) was available for further research; tissues were obtained from the lateral thigh while the patient was under general anesthesia. The biopsy sample from patient 1 was taken at the time of diagnosis and before any medication was started. Patient 2 underwent biopsy because of progression of serious disease despite intensive treatment (prednisone 0.7 mg/kg/day, methotrexate 20 mg/week, and pulse cyclophosphamide 750 mg/m²/month for 6 pulses).

Informed consent was obtained from all study participants and their parents. The local medical ethics review board approved the study.

**Immunohistochemistry of muscle biopsy tissues.** Acetone-fixed cryostat sections measuring 7 μm in thickness were used for immunoperoxidase studies. Hsp60 was localized with the mouse monoclonal antibody LK1, which has unique specificity for mammalian Hsp60 and is not cross-reactive with the bacterial counterpart. LK1 (a kind gift of P. van Kooten, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands) was applied to the sections overnight at 4°C, at a concentration of 3.2 μg/ml. This was followed by treatment with biotin-labeled donkey anti-mouse secondary antibody (Chemicon, Southampton, UK). Next, peroxidase-labeled streptavidin (Dako, Ely, UK) and Karnovsky’s diaminobenzidine medium (Sigma-Aldrich, Dorset, UK) were applied, followed by counterstaining with Harris’ hematoxylin (BDH Laboratory Supplies, Poole, UK).

**T cell activation in peripheral blood.** PBMCs from juvenile DM patients and healthy donors were examined for antigen-specific proliferative T cell responses. PBMCs were isolated by Ficoll density-gradient centrifugation (Pharmacia, Uppsala, Sweden) of heparinized blood. Cells were cultured in RPMI 1640 supplemented with 2 mM l-glutamine, 100 units/ml of penicillin/streptomycin (Gibco BRL, Gaithersburg, MD), and 10% (volume/volume) AB+ heat-inactivated (60 minutes at 56°C) human serum (Sanquin Blood Bank, Amsterdam, The Netherlands). Cells (2 × 10⁵ cells in 200 μl per well) were cultured in triplicate in round-bottomed 96-well plates (Nunc, Roskilde, Denmark) for 120 hours at 37°C in an atmosphere of 5% CO₂ with 100% relative humidity, in the presence of medium only, 10 μg/ml of human Hsp60 (Stressgen, Victoria, British Columbia, Canada), or 10 μg/ml of mycobacterial Hsp60 obtained from plasmid pRIB1300 as previously described (25). Tetanus toxoid (1.5 μg/ml; National Institute for Public Health and the Environment, Bilthoven, The Netherlands) was used as a positive control. A mouse class II–restricted epitope (ovalbumin [OVA] 323–339) was used as a negative control.

During the last 16 hours of culture, 1 μCi (37 kBq) of ³H-thymidine (ICN Biomedicals, Amsterdam, The Netherlands) was added to each well. Cells were harvested according to standard procedures. Radioactivity incorporated into the cells was measured by liquid scintillation counting and expressed as counts per minute. The magnitude of the proliferative response was expressed as a stimulation index, which was calculated as the mean cpm in cells cultured with antigen divided by the mean cpm in cells cultured without antigen. A stimulation index of ≥2 was considered a positive response.

**T cell activation in muscle biopsy tissues.** Muscle biopsy samples were stored in liquid nitrogen within 1 or 2 hours after collection. To study antigen-specific proliferative T cell responses in the inflammatory infiltrates, the biopsy samples were thawed and divided into pieces measuring 1–2 mm³. Samples were cultured in duplicate or triplicate (depending on material available) in round-bottomed 96-well plates for 120 hours at 37°C in an atmosphere of 5% CO₂ with 100% relative humidity in the presence of medium alone (as for PBMC culture), 20 μg/ml of human or mycobacterial Hsp60, or mouse class II–restricted epitope OVA³₂₃–₃₃₉, which was used as an irrelevant control.

After 96 hours, the remaining muscle tissue was removed, and 1 μCi (37 kBq) of ³H-thymidine was added to each well during the last 16 hours of culture. Cells were harvested according to standard procedures. Radioactivity incorporated into the cells was measured by liquid scintillation counting and expressed as cpm. The magnitude of the proliferative response was expressed as a stimulation index, which was calculated as described above.

**Cytokine production by multiplexed particle–based flow cytometry.** For analysis of cytokine production, cultures of T cells derived from peripheral blood and muscle tissue inflammatory cells from patients with juvenile DM were performed as described above. After 96 hours, cell culture supernatants were collected and stored at –80°C. Cytokine concentrations were measured with the Bio-Plex system using Bio-Plex Manager software version 4.0 (Bio-Rad Laboratories, Hercules, CA), which uses Luminex xMAP technology as previously described (26,27). For the study of PBMCs, the
cytokines interleukin-1β (IL-1β), IL-12, IL-13, IL-15, IL-10, tumor necrosis factor α (TNFα), and interferon-γ (IFNγ) were measured. For the study of the muscle biopsy samples, production of IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, IFNγ, TNFα, macrophage migration inhibitory factor (MIF), IL-8, interferon-inducible protein 10, monocyte chemotactractant protein 1 (MCP-1), and macrophage inflammatory protein 1α (MIP-1α) was measured in culture supernatants. The multiplex data (cytokine concentrations) were digitized to create a cytokine portrait enabling the complete spectrum of cytokines to be visualized.

**Analysis of messenger RNA (mRNA) by quantitative real-time reverse transcription–polymerase chain reaction (RT-PCR).** After 96 hours of stimulation with medium, human Hsp60, mycobacterial Hsp60, or OVA323–339, total RNA was isolated from inflammatory muscle infiltrates using Tripure isolation reagent (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. First-strand complementary DNA (cDNA) was synthesized from total RNA using Moloney murine leukemia virus reverse transcriptase (Promega, Madison WI) with 1 μg/μl of oligo(dT) and 0.5 mM dNTPs (both from Amersham Pharmaic Biotech, Uppsala, Sweden). The reaction mixture was incubated at 40°C for 90 minutes, followed by incubation at 70°C for 15 minutes. Levels of expression of IL-10 and IFNγ mRNA were determined by semiquantitative real-time RT-PCR using a LightCycler instrument with LightCycler reagent (Roche Diagnostics). In addition, β2-microglobulin (β2m) was analyzed as a housekeeping gene. The following primers were used: for IL-10, 5’-TGA-GAA-CAG-CTG-CAC-CCA-CTT-3’ (forward) and 5’-GCT-GAA-GGC-ATC-TCG-GAG-AT-3’ (reverse); for IFNγ, 5’-GCA-GAG-CCA-AAT-TGT-CTC-CT-3’ (forward) and 5’-ATG-CTC-CTC-GAC-CTC-GAA-AC-3’ (reverse); and for β2m, 5’-CCA-GGA-GAG-AAT-GGA-AAG-TC-3’ (forward) and 5’-GAT-GCT-GCT-TAC-ATG-TCT-CG-3’ (reverse). Results were normalized using β2m data derived from the same cDNA samples.

**Statistical analysis.** Statistical evaluation was performed using SPSS software, version 11.5 (SPSS, Chicago, IL). Group differences were analyzed with the Mann-Whitney U test. Paired data were analyzed with Wilcoxon’s signed rank test. P values less than 0.05 were considered significant.

**RESULTS**

**Immunohistochemistry of Hsp60 in juvenile DM.** Muscle biopsy samples from all 4 patients with juvenile DM showed expression of Hsp60 in most inflammatory cells, in degenerating and regenerating muscle fibers, and in the mural elements of small blood vessels (Figure 1). In muscle tissue from the healthy control subject, only some capillaries were stained (Figure 1). Thus, the expression of self Hsp60 was increased in muscle tissue from patients with juvenile DM.

**T cell activation of PBMCs in juvenile DM.** Next, we sought to determine whether this self Hsp60 could also be recognized by autoreactive T cells from patients with juvenile DM. To determine this, peripheral blood from 22 juvenile DM patients was tested for T lymphocyte proliferative responses to both human and mycobacterial Hsp60. T cell proliferative responses to human Hsp60 in PBMCs from patients with juvenile DM were significantly higher than those in PBMCs from healthy controls (Figure 2A). When stimulated with microbial Hsp60, no difference in the stimulation index between patients and healthy controls was detected. Also, no differences were found after stimulation with an irrelevant antigen (ovalbumin) and a positive control (tetanus toxoid).

Twelve of the juvenile DM patients had active disease (6 with new-onset disease) and 10 were in clinical remission. T cell recognition of human Hsp60 was stronger in patients during the inactive phase of disease: the frequency of responders to human Hsp60 (defined as a stimulation index ≥2) was much higher during disease remission than during active disease (44% versus 17%) (Figure 2B). Stimulation with microbial Hsp60 gave identical results: 60% of those in disease remission responded compared with 33% of those with active disease. No such differences were found after stimulation with control antigens. The differences in T cell proliferative responses to both juvenile DM patients and healthy controls were consistent in the few patients in which repeated testing was possible within 2
weeks after initial testing and without a major change in the clinical condition (data not shown).

Cytokine production by PBMCs after stimulation with Hsp60 in juvenile DM. We also tested for cytokine production induced by Hsp60 in PBMCs from 15 patients and 6 healthy controls (Table 2). Because of the small volume of patient samples, we performed the analysis of all secreted cytokines at 1 time point, being aware that the optimal time point for cytokine production following stimulation may differ between cytokines (27). We found significantly increased secretion of IL-1β, TNFα, and IL-10, but not IFNγ, IL-2, IL-13, and IL-15, following in vitro activation of PBMCs with human and microbial Hsp60 as compared with cytokine production by cells cultured without antigen. In addition, we found that after stimulation with human Hsp60, production of IL-1β, TNFα, and IL-10 was significantly increased in patients with active disease but not in those whose disease was in remission. In contrast, stimulation with microbial Hsp60 resulted in increased production of IL-1β, TNFα, and IL-10 during both active disease and disease remission.

Comparisons of cytokine secretion between juvenile DM patients and healthy controls showed that there was more IL-10 (*P* = 0.002) and less TNFα (*P* = 0.062) produced by healthy control PBMCs after stimulation with human Hsp60. Comparable results were found after stimulation with microbial Hsp60 (*P* = 0.016 and *P* = 0.036, respectively). Individual results are shown in Figure 3.

Table 2. Cytokine production induced in PBMCs from 15 of the juvenile DM patients and 6 healthy control subjects after stimulation with human Hsp60, microbial Hsp60, or medium only*

<table>
<thead>
<tr>
<th>Juvenile DM patients</th>
<th>IL-1β, mean ± SEM pg/ml</th>
<th>IL-10, mean ± SEM pg/ml</th>
<th>TNFα, mean ± SEM pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (n = 15)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Hsp60</td>
<td>780 ± 1,743†</td>
<td>22 ± 56†</td>
<td>131 ± 273†</td>
</tr>
<tr>
<td>Mycobacterial Hsp60</td>
<td>2,766 ± 2,003†</td>
<td>188 ± 309†</td>
<td>1,144 ± 1,813†</td>
</tr>
<tr>
<td>Medium only</td>
<td>58 ± 123</td>
<td>13 ± 25</td>
<td>10 ± 18</td>
</tr>
<tr>
<td><strong>Active juvenile DM (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Hsp60</td>
<td>1,289 ± 2,290†</td>
<td>13 ± 2†</td>
<td>130 ± 228†</td>
</tr>
<tr>
<td>Mycobacterial Hsp60</td>
<td>3,298 ± 1,985†</td>
<td>226 ± 410†</td>
<td>1,551 ± 2,419†</td>
</tr>
<tr>
<td>Medium only</td>
<td>12 ± 17</td>
<td>3 ± 4</td>
<td>3 ± 2</td>
</tr>
<tr>
<td><strong>Juvenile DM in remission (n = 7)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Hsp60</td>
<td>198 ± 476</td>
<td>33 ± 81</td>
<td>132 ± 337</td>
</tr>
<tr>
<td>Mycobacterial Hsp60</td>
<td>2,158 ± 1,989†</td>
<td>145 ± 151†</td>
<td>679 ± 610†</td>
</tr>
<tr>
<td>Medium only</td>
<td>110 ± 170</td>
<td>24 ± 34</td>
<td>18 ± 24</td>
</tr>
<tr>
<td><strong>Healthy controls (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Hsp60</td>
<td>313 ± 245</td>
<td>161 ± 94</td>
<td>108 ± 70</td>
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<tr>
<td>Mycobacterial Hsp60</td>
<td>565 ± 291</td>
<td>325 ± 95</td>
<td>287 ± 150</td>
</tr>
<tr>
<td>Medium only</td>
<td>9 ± 15</td>
<td>30 ± 17</td>
<td>4 ± 6</td>
</tr>
</tbody>
</table>

* PBMCs = peripheral blood mononuclear cells; DM = dermatomyositis; IL-1β = interleukin-1β; TNFα = tumor necrosis factor α.
† *P* ≤ 0.05 versus cells stimulated with medium only.
T cell reactivity to Hsp60 in muscle tissues from juvenile DM patients. Finally, we wanted to investigate whether self Hsp60 could also be recognized by autoreactive T cells in the inflammatory infiltrates in muscle biopsy samples from juvenile DM patients. We analyzed muscle tissue from 2 patients who showed typical histopathologic features of perifascicular atrophy with inflammatory infiltrates. After stimulation for 96 hours with medium, human Hsp60, microbial Hsp60, and ovalbumin, 40–97% of the mononuclear cells in the culture stained positive for CD3 by fluorescence-activated cell sorter analysis. We were able to demonstrate T cell proliferative responses to human and microbial Hsp60 in muscle tissue–derived mononuclear cells (MDMCs) from the 2 juvenile DM patients (Figure 4A). These MDMC responses were in the same range as the responses of the PBMCs.

In addition, cytokine production was measured by multiplexed particle–based flow cytometric analysis of supernatants after stimulation of MDMCs from these 2 patients with human Hsp60. There was increased production of IL-6, IL-17, IFNγ, MIF, and IL-8, but no differences in production of the other cytokines tested (see Patients and Methods) (data not shown). IFNγ and MIF levels were increased in mononuclear cells derived from both biopsy samples, but only in patient 2 was there a marked increase in all other proinflammatory cytokines tested. Remarkably, patient 2 had a chronic active disease pattern, which was resistant to a combination of potent immunosuppressive agents.

We also analyzed the Hsp60-specific MDMCs for IFNγ and IL-10 mRNA (Figure 4B). We found an elevated ratio of IL-10 to IFNγ in response to human Hsp60 in patient 1, who had a mild pattern of disease.

DISCUSSION

For a long time, the focus in autoimmune research has been to find the specific self antigen that triggers the disease. It is likely, however, that human autoimmune diseases, given their heterogenic nature, may start out as a reaction to probably many different self antigens. Once the disease progresses, the immune response will quickly spread to other self-antigenic targets. Thus, it may be advantageous to focus not on disease-causing antigens, but rather, on antigens that
play an immunomodulatory role in the perpetuation of the inflammation (5). Such “spreading” self antigens could be used as diagnostic markers of disease activity or as targets for antigen-specific immune therapy.

Juvenile DM is a systemic autoimmune disease with an unknown pathogenesis (1). Relatively little is known about self antigens involved in juvenile DM. Only recently, the first epitope was identified in juvenile DM. This was an epitope of the *Streptococcus pyogenes* M5 protein, which shares homology with human skeletal myosin and can elicit proinflammatory responses in skeletal muscle tissue (28).

The present study is the first to show that Hsp60 is a disease-related autoantigen in juvenile DM. At the site of inflammation in affected muscle tissue, we found increased expression of Hsp60. T cell responses to self Hsp60 were also present in PBMCs from children with juvenile DM, as well as in MDMCs from the inflamed muscle tissue of juvenile DM patients. The question then arises, What role does this autoreactive T cell response play in the course of juvenile DM?

HSPs acting as stress proteins play a dual role in the inflammatory process. First, HSPs can aggravate the innate immune response through Toll-like receptor signaling (6). Second, HSPs can also activate the adaptive immune response, which may have a regulatory effect. More evidence for the latter role comes from studies in both experimental models and humans with arthritis, atherosclerosis, and type 1 diabetes mellitus, diseases in which reactivity to Hsp60 has been associated with down-regulation of the autoimmune disease processes (6,7,11). T cells cross-reactive with self Hsp60, which are tolerated at the mucosal surfaces of the gastrointestinal tract after contact with microbial Hsp60, can home toward Hsp60 expressed at sites of inflammation (7). A similar mechanism may also be applicable to inflammatory muscle disease. Indeed, Hsp60 is expressed in higher amounts in injured muscle tissue. Following activation, self-HSP–reactive T cells show a mixed phenotype of both effector and regulatory T cells, as illustrated by the antigen-specific secretion of both proinflammatory cytokines and the antiinflammatory cytokine IL-10 by PBMCs.

Our findings, however, underscore a potential immune regulatory role of Hsp60-specific T cells in juvenile DM. First, T cell proliferation in response to...
Hsp60 was stronger in patients during the inactive phase of disease. This is consistent with findings in patients with juvenile idiopathic arthritis, in whom disease remission is preceded by a proliferative response to Hsp60 (14). Second, the mixed pattern of cytokine secretion (IL-1β, TNFα, and IL-10) in response to human Hsp60 in patients with active disease seems to reflect the homing of both effector and regulatory T cells toward sites of inflammation. The outcome of this balance between activation and regulation may very well be essential for the chronic persistence or clearance of the inflammatory process in the muscle. This is consistent with the Hsp60-specific cytokine profiles demonstrated in our study of muscle biopsy samples from 2 juvenile DM patients: both biopsy specimens were obtained during active disease and showed a predominantly proinflammatory cytokine profile. However, a clearly higher ratio of IL-10 to IFNγ was found in muscle tissue from patient 1 as compared with that from patient 2, which suggests a more favorable outcome in the balance of regulatory and effector T cells. The clinical course of these patients seems to reflect these results: patient 1 had a mild disease course, whereas patient 2 had a serious, chronic, continuous disease course.

Taken together, the findings of this study demonstrate that T cell responses to human Hsp60 play an active role in the control of inflammation in juvenile DM. Thus, therapy aimed at expansion of Hsp60-specific T cells that have a regulatory phenotype in patients with juvenile DM could contribute to disease remission. It is conceivable that other immunodominant antigens might also serve as bystander epitopes in juvenile DM. One of the most interesting antigens in this context is Hsp70, since previous studies have suggested a muscle-protective role of Hsp70.

Our studies were based on the use of whole proteins, which may be a confounding factor. Different epitopes from the same protein may have different, and sometimes even reverse, effects on the quality of T cell responses (15). We will have to analyze further the specific Hsp60 epitope recognition in order to identify T cells that have regulatory capacity. These distinct epitopes can then be used as biomarkers of disease activity and as candidate antigens for immunomodulatory therapy.

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AUTHOR CONTRIBUTIONS

Drs. Elst and Prakken had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Elst, Albani, Kuis, Prakken.

Acquisition of data. Elst, Klein, de Jager, Kamphuis, Wedderburn.

Analysis and interpretation of data. Elst, Prakken.

Manuscript preparation. Elst, Kamphuis, Wedderburn, van der Zee, Albani, Kuis, Prakken.

Statistical analysis. Elst, de Jager, Prakken.

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