T-Cell Apoptosis in Inflammatory Neuromuscular Disorders Associated With Human Immunodeficiency Virus Infection

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Background: Apoptosis is one of the major mechanisms of CD4+ T-cell depletion in human immunodeficiency virus (HIV) infection. T cells in human inflammatory myopathies in HIV-negative individuals rarely undergo apoptosis. Currently, there is no information available concerning the fate of T cells in HIV-associated myositis and polyneuropathies.

Objective: To investigate whether apoptosis occurs in inflammatory lesions of muscle and nerve biopsy specimens of untreated HIV-positive patients with neuromuscular disorders.

Methods: T-cell apoptosis was investigated in muscle and nerve specimens from 12 patients with HIV-associated polymyositis and 8 patients with HIV-associated inflammatory polyneuropathy. These were compared with specimens from 36 HIV-negative patients with other inflammatory myopathies and from 18 patients with inflammatory polyneuropathies. Apoptosis was assessed according to morphological criteria and with the use of in situ labeling methods.

Results: In none of the HIV-associated disorders did we observe a substantial proportion of apoptotic T cells as assessed by nuclear morphological findings and in situ labeling techniques. Fas expression was up-regulated only in a few inflammatory cells. Positive labeling for Fas ligand was not associated with increased apoptosis of surrounding T cells. Nuclei of degenerating muscle fibers and macrophages did not show morphological signs of apoptosis and were not labeled by the tailing reaction.

Conclusions: Similar to their idiopathic counterparts, in HIV-related polymyositis and inflammatory neuropathy, T-cell inflammation is not cleared by apoptosis. The observations are consistent with the non-self-limited nature of endomyial or endoneural inflammation and suggest that in HIV-positive patients, the T-cell elimination is differentially regulated in the lymphoid organs as compared with neuromuscular tissues.

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PATIENTS AND METHODS

PATIENTS

Diagnostic muscle biopsy specimens were obtained from 12 HIV-positive patients with polymyositis who gave informed consent. Diagnostic sural nerve biopsy specimens were taken from 8 HIV-positive and 18 HIV-negative patients with clinical and electrophysiological signs of inflammatory neuropathy. Patients with HIV-related polymyositis and polynuereitis were in different stages of the disease according to the Centers for Disease Control and Prevention classification, most being in clinically manifest acquired immunodeficiency syndrome (AIDS) (stage III). Most patients were untreated at the time the biopsy specimen was taken, but some patients with polymyositis had received therapy with nucleoside analogs. One part of the muscle or nerve specimen was snap frozen in isopentane prechilled with liquid nitrogen and processed for cryosections. Other parts were fixed by immersion in freshly prepared paraformaldehyde and routinely embedded in paraffin as described previously or fixed in 3.6% isotonic glutaraldehyde at pH 7.4 for at least 4 hours and processed for paraffin or cryosections and semithin sections. Muscle and nerve biopsy specimens were derived from all 3 institutions participating in this study.

IMMUNOCYTOCHEMISTRY AND HISTOLOGICAL ANALYSIS

Five-micrometer cryosections of muscle and nerve specimens were mounted on poly-L-lysine–coated slides and fixed for 10 minutes in acetone at −20°C. Sections were blocked with porcine serum (Biochrom KG, Berlin, Germany) for 20 minutes. Cellular infiltrates were characterized in several sections by incubation with monoclonal antibodies recognizing CD68 (dilution, 1:100; Dako, Hamburg, Germany), CD4 (Leu 3a; dilution, 1:20; Becton Dickinson Co, Heidelberg, Germany), and CD8 (Leu 2a; dilution, 1:50; Becton Dickinson Co) for 1 hour at room temperature. In addition, IgM monoclonal antibodies recognizing Fas antigen (dilution, 1:100; Upstate Biotechnology Inc, Lake Placid, NY) or Fas ligand (concentration, 0.3 mg/mL; Transduction Laboratories, Lexington, Ky) were used. Subsequently, the avidin-biotin complex (ABC) detection system (Dako) and 3,3’-diaminobenzidine as peroxidase substrate were used. Endogenous peroxidase activity was suppressed by incubating the sections for 10 minutes in 3% hydrogen peroxide before adding the ABC reagent. Finally, sections were counterstained with hematoxylin, dehydrated, and mounted in embedding medium (Entuk; Kindler, Freiburg, Germany).

The number of labeled lymphoid and muscle cells was evaluated in transverse sections in 5 fields of 1 mm² each at ×100 primary magnification per marker. The percentage of cells undergoing apoptosis was assessed by means of in situ tailing and morphological criteria: cell shrinking with nuclear chromatin condensation, collapse of the chromatin into patches along the nuclear membrane, and, finally, formation of apoptotic bodies.9,10

IN SITU TAILING

In situ tailing was performed on paraffin-embedded and frozen material as described previously. Briefly, after deparaffinization, 5-µm tissue sections were treated with chloroform and washed in 0.05-mol/L Tris buffer. Frozen sections were dried and fixed as described above. Sections were then incubated for 1 hour with 50 µL of a reaction mixture containing terminal transferase and fluorescein-labeled nucleotides (In Situ Cell Death Detection Kit, Boehringer, Mannheim, Germany). The reaction was stopped by adding 0.5-mol/L EDTA. Sections were then treated for 1 hour with an alkaline fluorescein-labeled antifluorescein antibody (Boehringer) used as a chromogen. After tailing, the same sections were stained for T cells by means of the monoclonal anti-CD45 antibody UCHL-1 (dilution, 1:100; Dako) on paraffin-embedded material and the anti-CD3 monoclonal antibody Leu4 (dilution, 1:50; Becton-Dickinson Co) on frozen sections, visualized with the ABC detection system conjugated with alkaline phosphatase and developed with a fast red salt (Sigma Chemical Co, Deisenhofen, Germany).

As positive controls for the tailing reaction, we used inflamed spinal cord from experimental encephalomyelitis, where a high proportion of T-cell apoptosis occurs.

Range of T-Cell Infiltration and Apoptosis in Different Forms of Inflammatory Neuropathies and Myopathiesa

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Patients</th>
<th>T Cells/mm², Range (Median)</th>
<th>CD4/CD8 Ratio, Range (Median)</th>
<th>Rate of Apoptosis, Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-related polymyositis</td>
<td>12</td>
<td>9.5-321 (66)</td>
<td>0.3-1.2 (0.61)</td>
<td>0 per 1000</td>
</tr>
<tr>
<td>HIV-associated polynuereitis</td>
<td>8</td>
<td>57-199 (141)</td>
<td>0.1-0.66 (0.4)†</td>
<td>0 per 1000</td>
</tr>
<tr>
<td>Idiopathic polymyositis</td>
<td>12</td>
<td>11-214 (47)</td>
<td>0.62-3.5 (1.2)†</td>
<td>0-3 per 1000</td>
</tr>
<tr>
<td>Idiopathic polynuereitis</td>
<td>5</td>
<td>33-900 (68)</td>
<td>0.42-0.59 (0.47)</td>
<td>0 per 1000</td>
</tr>
<tr>
<td>CIDP</td>
<td>13</td>
<td>6-35 (15)</td>
<td>0.25-5.0 (1.45)</td>
<td>0 per 1000</td>
</tr>
</tbody>
</table>

a HIV indicates human immunodeficiency virus; CIDP, chronic inflammatory demyelinating polynuereiopathy.
†CD4/CD8 differentiation was possible in only 4 patients with HIV-associated neuritis and 7 patients with polymyositis. Patients with polymyositis were examined in a previous study.

Diagnosis of HIV-related polymyositis or HIV-related neuritis was established on the basis of characteristic virus itself influences the elimination of T cells from the respective tissues. Furthermore, we searched for the expression of proapoptotic signals such as the Fas molecule and its ligand.

RESULTS

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A and B, CD8 immunolabeling of human immunodeficiency virus (HIV)–related myositis (A) and double-labeling with T-cell antibodies (red) and in situ tailing for demonstration of DNA fragmentation (black) (B). No apoptotic T cells can be observed. C through E, Immunocytochemical detection of Fas (C), Fas ligand (D), and CD8 (E) in serial sections from a patient with HIV-related myositis. Note that cells adjacent to Fas ligand–positive cells do not exhibit apoptotic morphological characteristics. F and G, Immunocytochemical detection of CD8 (F) and double-labeling with T-cell antibodies (red) and in situ tailing for demonstration of DNA fragmentation (black) in sural nerve from an HIV-positive patient (G). There is no DNA fragmentation indicative of apoptosis. Bar indicates 10 µm.
clinical, serological, morphological, and immunopathological features. The same criteria were used for diagnosis of idiopathic inflammatory myopathies, polymyositis, and chronic inflammatory demyelinating polyneuropathy.

In all cases with HIV-associated myositis there was ongoing inflammation with endomyosial infiltrates invading nonnecrotic muscle fibers. Mean density of inflammatory infiltrates and the ratio of CD4- and CD8-positive T cells in muscle and nerve specimens are given in the Table. T cells with apoptotic morphological characteristics could not be observed (Figure, A). The absence of DNA fragmentation typical of T-cell apoptosis was also demonstrated by in situ tailing (Figure, B). Up-regulation of Fas antigen was detectable on mononuclear cells (Figure, C) and muscle fibers (not shown). In some specimens, strong expression of Fas ligand was seen on mononuclear cells (Figure, D). No apoptotic T cells or myonuclei were noted adjacent to Fas ligand–positive cells as observed by morphological examination (Figure, E) and in situ tailing (not shown).

Similarly, neither in HIV-positive (Figure, F and G) nor in HIV-negative patients with inflammatory neuropathies could apoptotic T cells be detected by in situ tailing.

Apoptosis has been suggested to be one of the major mechanisms of T-cell depletion in the peripheral blood and in lymph nodes of HIV-infected individuals. Not only peripheral blood lymphocytes but also lymph node cells undergo apoptosis more frequently in HIV-infected patients than in normal subjects. We observed virtually no apoptosis in HIV-related myositis or in inflammatory neuropathies occurring through the course of AIDS. Furthermore, degenerating muscle fibers in HIV-related myositis preferentially exhibited morphological signs of necrosis. Thus, HIV-related myositis behaved similarly to other idiopathic inflammatory myopathies in nonimmunocompromised patients, where the absence of T-cell apoptosis has been reported recently.

Acute or chronic inflammatory polyneuropathies, mononeuritis multiplex, and HIV-associated polymyositis that may develop during the course of HIV infection are thought to have an autoimmune pathogenesis. This is supported by the scarcity of HIV-infected mononuclear cells within the endomyosial infiltrates (only scarce macrophages are HIV positive), the resistance of mature muscle fibers to infection with HIV, and the histopathological findings resembling those in HIV-negative patients. Furthermore, some of the HIV-associated diseases may respond to immunosuppressive and immunosuppressive treatments. Studies in the brains of HIV-infected patients suggest that qualitative features of the virus, rather than increased virus load, are responsible for the clinical phenotype.

Several mechanisms have been discussed to explain the lack of T-cell apoptosis in inflammatory myopathies. Among those are a lack of proapoptotic signals and the diversity of involved autoantigens recognized by the inflammatory cells. Muscle tissue may not possess those specialized mechanisms of immune protection that appear to operate in other tissues, such as the eye and the central nervous system (for review, see Gold et al). It is unclear, however, whether in HIV-positive patients there are unknown protective factors that prevent apoptosis of the T cells in the microenvironment but not in the lymph nodes or whether peripheral blood lymphocytes primed for apoptosis are not those that infiltrate the muscle tissue. Alternatively, the absence of apoptosis in polymyositis and in inflammatory polyneuropathies in both HIV-positive and HIV-negative patients suggests that proapoptotic factors are not expressed in these tissues. It is also likely that T-cell apoptosis occurs early in the autoimmune T cells, when the degree of inflammation is prominent, as observed in experimental autoimmune neuritis, an animal model for human immunoinflammatory neuropathies, and not during the chronic but active stage of the disease as in the patients we studied.

Possible proapoptotic factors in AIDS include up-regulation of Fas or Fas ligand and tumor necrosis factor α, dysregulation of cysteine or serine protease inhibitors, the transactivating protein, and cytokines such as lymphotoxin, interleukin 4, and interleukin 10. In our specimens we found that Fas and its ligand were expressed and cytokines such as interferon gamma and tumor necrosis factor α are variably up-regulated in HIV-related polymyositis, indicating that the scarcity of T cells undergoing apoptosis is not likely to be caused by the absence of these signals. The difference in tissue-specific elimination of activated autoreactive lymphocytes in HIV-positive patients may relate to compartmental breakdown of immunoregulating factors that lead to the development of autoimmunity, resulting in inflammatory myopathies or neuropathies.

The lack of spontaneous apoptosis in inflammatory myopathies and most neuropathies in AIDS and in nonimmunocompromised persons may have pathological implications. In contrast to multiple sclerosis and experimental autoimmune encephalomyelitis, where inflammatory lesions are cleared even without specific treatment, here the disease course is often relentlessly progressive unless immunosuppressive treatment is instituted.

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REFERENCES

2. Schmied M, Breitschopf H, Gold R. Apoptosis of T lymphocytes in experimental autoimmune encephalomyelitis: evidence for programmed cell death as a mecha-


