In Situ Immunophenotype of the Inflammatory Infiltrate in Eosinophilic Fasciitis

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ABSTRACT. Objective. Eosinophilic fasciitis (EF) is histologically characterized by a fibrous and inflammatory thickening of subcutaneous septal-fascial-perimysial collagenous scaffold. This study aims to define the immunophenotype of inflammatory cells of fascia and muscle underlying the in situ immune response in EF.

Methods. In 11 cases of EF, we determined the phenotype of inflammatory cells, expression of MHC class I and class II antigens, and C5b9 membranolytic attack complex (MAC) deposits by immunohistochemistry analysis of fascia tissue. Muscle biopsies from 9 patients with active dermatomyositis and 5 with active polymyositis were used as controls.

Results. In all patients but one, the inflammatory infiltrate was mainly composed of macrophages associated with CD8+ T lymphocytes (CD4/CD8 ratio < 1) and few eosinophils. Cytotoxic properties were found in 14% of CD8+ T lymphocytes, as shown by granzyme B expression. MHC Class I antigens were overexpressed (5/7) by muscle fibers, with a paratrabecular reinforcement in 4 cases. MHC class II antigens were not expressed by muscle fibers except in one case. C5b9 MAC deposits were not detected.

Conclusion. Our in situ characterization of inflammatory infiltrate demonstrates the predominancy of macrophages and CD8+ T lymphocytes. Some of these CD8+ T lymphocytes contain granzyme B, thus suggesting a cytotoxic cellular immune response in EF, which could be triggered by infectious or environmental agents. (J Rheumatol 2003;30:1811–5)

Key Indexing Terms: SHULMAN IMMUINOPTHENOTYPE EOSINOPHILIC FASCITIS FASCITIS-PANNICULITIS INFLAMMATORY INFILTRATE

The pathophysiology of the disease is still unknown. However, immune-mediated mechanisms appear to play a pivotal role6-8. The immune origin of this disease is supported by the successive detection of elevated immunoglobulins and circulating immune complexes in patients with active EF and finally the occurrence of EF in chronic graft-versus-host disease6,8. In addition, autoimmune mechanisms have been proposed on the basis of the association of EF with other autoimmune disorders7.

Interestingly, in their attempt to define the cytokine network potentially playing a role in the cascade of events leading to tissue fibrosis, Viallard, et al showed the overexpression of type 1 and type 2 cytokines from peripheral blood cells9. In addition, they concluded that a thorough immunophenotypic characterization of the local inflammatory infiltrate was mandatory to understand the pathophysiology of EF.

We characterized the inflammatory infiltrate and demonstrated the predominance of macrophages and CD8+ T lymphocytes. Cytotoxic properties were found in 14% of CD8+ T lymphocytes, as shown by granzyme B expression. Our results suggest a cytotoxic cellular immune response in EF that could be triggered by infectious or environmental agents.

MATERIALS AND METHODS

Patients. Eleven patients were selected from a series of 25 patients seen...

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over a 19-year period (1992 to 2000) in a single institution (University Hospital, Nantes, France) for the first manifestations of EF. The criteria used for inclusion were 4-fold: (1) clinical: the common criteria were painful edema and subcutaneous sclerotic induration without Raynaud’s phenomenon or a scleroderma capillaroscopic pattern; (2) biological: inflammatory syndrome, immunoglobulin overproduction, and eosinophilia; (3) pathological: exclusion of any other pathological entities belonging to the fascitits-panniculitis syndrome such as L-tryptophan ingestion, autoimmune disease (lupus, scleroderma), or the inflammatory myopathies; and (4) availability of frozen tissue (i.e., fascia and muscle samples) for immunohistochemical studies.

Clinicopathological data were retrospectively obtained from medical and pathological reports. All patients were examined by the same physician (J-MM). Biopsies were performed before any steroid or immunosuppressive therapy.

Pathological studies. A full thickness biopsy including skin, fascia, and muscle was performed from a clinically involved site for diagnostic purposes. Three muscle samples were rapidly snap frozen in isopentane precooled in liquid nitrogen, or fixed in formalin and in 2.5% glutaraldehyde. Frozen sections of muscle specimens were submitted to the following stainings and histochemical reactions: hematoxylin-eosin (H&E), modified Masson trichrome, NADH-tetrazolium reductase, myosin ATPase activity with preincubations at pH 4.3, 4.6 and 9.4, acid phosphatase, nonspecific esterases, periodic acid Schiff reaction, and Oil Red O, following standard methods. Skin biopsies were stained with H&E. Full thickness skin-to-muscle biopsies were examined histologically by 3 independent pathologists.

Immunohistochemical studies. Immunohistochemical studies were performed in an Autostainer apparatus (Immunootech, Marseille, France), by using a streptavidin-biotin peroxidase method on acetone-fixed frozen sections, according to the manufacturer’s instructions (LSAB kit, Dako, Paris, France). The following primary antibodies were used: CD3 (clone UCHT1, diluted 1:150; Dako), CD4 (clone BL4, diluted 1:80; Immunootech), CD8 (clone B9-2, diluted 1:100; Immunotech), CD20 (clone L26, diluted 1:100; Dako), CD11b (clone BEAR 1, diluted 1:50; Immunotech), CD14 (clone RM052, diluted 1:100; Immunotech), CD68 (clone PGM1, diluted 1:60; Immunotech), C5b9 (Clone eE1, diluted 1:500; Dako), HLA CL1 (clone W6/32, diluted 1:150; Dako); HLA CL2 (clone B12, diluted 1:100; Immunotech). The chromogen used was DAB (3,3’-diaminobenzidine tetrahydrochloride) and tissue sections were counterstained with hematoxylin. Appropriate negative controls were used throughout. The control disease group for immunohistochemical studies included muscle biopsies from 9 patients with dermatomyositis and 5 with polymyositis.

To more precisely characterize the cytotoxic properties of CD8+ T lymphocytes, immunohistochemistry was performed on paraffin-embedded sections after antigen retrieval using the following antibodies: anti-granulysin B (NCL-GRANB, diluted 1:100; Novocastra, Tebu, Le Perray en Yvelines, France), anti-TIA-1 (clone 2G9, diluted 1:100; Immunotech), and CD56 (clone 123C3, diluted 1:20; Zymed Clinisciences, Montrouge, France). For this study, 7 cases, with a marked inflammatory infiltrate were selected.

For each sample, we performed: (1) a semiquantitative analysis of the inflammatory infiltrate [slight (+), moderate (++), marked infiltrate (+++)]; (2) an immunophenotypic analysis in a 2 step method: first, a quantitative analysis of the mononuclear cell subsets scoring positive with the antibodies was performed [no staining (0); 0–10% positive cells (+); 10–30% positive cells (++); 30–60% positive cells (+++); > 60% positive cells (++++)], second, the CD4/CD8 T lymphocytes ratio and the macrophage/CD3+ T lymphocyte ratio were determined; and (3) an analysis of the expression of MHC class I and/or class II antigens, as well as the presence of C5b9 membranolytic attack complex (MAC) deposits on vascular walls, performed in 7 cases.

Ultrastructural studies. An ultrastructural study was performed in 7 muscle biopsies according to standard methods.

RESULTS

Pathological findings. Lesions of fasciitis-panniculitis defined as a fibrous and inflammatory thickening of subcutaneous septal-fascial-perimysial collagenous scaffold with minimal muscle lesions were observed in 9 out of 11 cases (Figure 1A and B).

The inflammatory infiltrate was mild (+) to moderate (++) composed mainly of macrophages, lymphocytes, and a few eosinophils. In 2 out of 11 cases, numerous eosinophils were noted. The infiltrate was randomly distributed within the collagen tissue or in a perivascular situation (Figure 1A and B).

No striking histochemical changes such as perifascicular atrophy or specific fiber atrophy were noticed. In only 2 cases, a myotic process was obvious, i.e., necrosis and regeneration of muscle fibers, rounding of myocytes, and increased variation of diameter associated with endomysial inflammation. In the other cases, the muscle lesions were minimal and nonspecific, with irregular-sized, segmented fibers or nuclear centralization.

Pathological changes were noted in the skin in 8 out of 11 cases, consisting of hypodermic or dermohypodermic sclerosis without skin appendage involvement. These changes were associated with a patchy perivascular or a more diffuse inflammatory infiltrate. The extension and intensity of the lesions varied from one case to another. In 2 out of 11 cases, they appeared as a slight perivascular dermatitis. Epidermotropism was noted in 6 out of 11 cases.

Immunohistochemical findings. Table 1 summarizes the immunohistochemical data. Quantification of the infiltrate revealed that the majority of cells consisted of macrophages (CD14+, CD11+, CD68+ cells) followed by CD3+ CD8+ T lymphocytes and a lower number of CD3+ CD4+ T lymphocyte-cells (Table 1, Figure 1C and D). In 8 out of 11 cases, macrophages were predominant. Among the CD3+ T lymphocyte population, CD8+ T cells were predominant (CD4+/CD8+ < 1) in 7 out of 11 cases (Table 1, Figure 1D). The proportion of CD8+ and CD4+ T cells was equal in 3 out of 11 cases. By contrast, in one case, CD4+ T cells were predominant (CD4+/CD8+ > 1).

To more precisely characterize the cytotoxic features of CD8+ T cells, an immunohistochemical analysis was performed in 7 cases on paraffin sections using anti-TIA-1 and anti-granulysin B antibodies. TIA-1 was detected in 43 ± 7% of CD8+ T cells and granulysin B in 14 ± 3% of CD8+ T cells (Figure 1E and F). CD56+ natural killer (NK) cells were rare. B lymphocytes were very rare or absent.

Immunoreactivity for MHC class I antigens was detected on the surface and the cytoplasm of muscle fibers in 5 out of 7 cases. The intensity of the staining was stronger on the muscle fibers located in the paratrabecular area close to the inflammatory infiltrate (Figure 1G). In all cases, MHC class I antigen expression was consistently observed in necrotic, degenerating, and regenerating muscle fibers (Table 1).
Figure 1. In situ immunophenotype of the inflammatory infiltrate in EF. In EF, the inflammatory infiltrate was located in fibrotic thickening fascia, with minimal muscle lesions (H&E, A and B). The inflammatory infiltrate was composed of CD68+ macrophages (C) and numerous CD8+ T lymphocytes (D). Some of the cells of the CD8+ infiltrate expressed TIA-1 (E) and granzyme B proteins (F), thus confirming their cytotoxic properties. Muscle fibers expressed MHC class I antigens with a paratrabecular reinforcement (G) and MHC class II antigens, with a slight and focal staining (H). (A, original magnification ×200; B to H, original magnification ×400).
cytes. Our findings show that the inflammatory infiltrate is composed of activated peripheral blood mononuclear cells, as evidenced by the expression of MHC class II antigens, identified by an anti-HLA-DR antibody. Our immunophenotype and MHC antigen expression analysis of 11 patients with eosinophilic fasciitis (EF) revealed that the inflammatory infiltrate was mainly composed of macrophages and CD8+ T lymphocytes, with the CD4/CD8 ratio being < 1 in most cases.

The increase in CD8+ lymphocytes reported here is in line with the increases of interleukin 2 (IL-2) and interferon-γ (IFN-γ) production found in peripheral blood, two Th1 cytokines produced in EF and known to stimulate CD8+ T lymphocyte proliferation. It also suggests that a cytotoxic response is likely to play an important role in the pathogenesis of EF. The cytotoxic properties of CD8+ T cells were confirmed by the presence of granzyme B in 14% of CD8+ T lymphocytes. Interestingly, a higher proportion of CD8+ T cells expressed TIA-1 antigen than granzyme-B antigen. This discrepancy can be explained by the fact that TIA-1 is expressed by both activated and non-activated T cells and NK cells, whereas granzyme B is expressed only by activated T cells and NK cells.

Granzyme B is a powerful pro-apoptotic granzyme, as it shares with caspases the capacity to cleave the acidic residues of proteins, especially aspartate acid residues. Its principal function is to induce death in virus-infected cells and other potentially harmful cells. Granzyme B expression results from the induction of a cytotoxic phenotype upon exposure of T lymphocytes to antigen or other type of stimulation. Granzyme B is stored within specialized “secretory lysosomes” and is discharged from cytotoxic T lymphocytes with the other granule toxin perforin following receptor-mediated conjugate formation with a target cell.

The cytotoxic lesions may also involve eosinophils, which are often detected in EF. Interestingly, alterations have been observed in their granule contents by ultrastructural analysis. Eosinophils are known to produce toxic inflammatory mediators including major basic protein and eosinophil cationic protein. Further damage is caused by hydrogen peroxide and halide acids, which are generated by eosinophil peroxidase, and by superoxide, which is generated by the respiratory-burst-oxidase pathway. Their recruitment is the result of overproduction of IL-5, a cytokine produced by Th2 cells activated by antigen-presenting cells. Chemokines produced by resident cells also participate in the recruitment of eosinophils. Finally, in addition to their role in recruiting eosinophils, macrophages participate in T lymphocyte activation. Altogether, these data, and our observation of numerous macrophages in EF lesions, point to a pivotal role of macrophages, as suggested by Barnes, et al.

The mechanisms of cytotoxic CD8+ T lymphocytes have been described in polymyositis, where the muscle lesions implicate a MHC class I-restricted cytotoxic process. In this context, we examined the expression of MHC class I antigens, which are expressed by the CD8+ T lymphocytes of target cells. It is known that nonpathological muscle fibers usually do not express MHC class I antigens. Interestingly, in our study, muscle fibers showing no lesions of necrosis expressed MHC class I antigens in 70% of EF cases. In contrast to polymyositis, this may indicate a different target in EF, such as fascia interstitial cells.

The MHC class I antigen expression of muscle fibers, when present, was diffuse and tended to be more intense in the paratrabecular areas where the inflammatory infiltrate was present. The MHC class I expression by muscle fibers could be a “bystander” event. MHC class I antigen expression could be upregulated by cytokines secreted by activated T cells or macrophages, as reported in various pathological states.

Altogether, our results lead to the hypothesis that Shulman syndrome, or EF, is an immune disorder, characterized by a fibrous thickening of the subcutaneous septal...
fascial-perimysial collagenous scaffold (or “fascitis-panniculitis syndrome”) with minimal muscular lesions. The phenotypic changes observed in muscle fibers might be a “bystander” event due to IL-2 and IFN-γ overproduction. In addition, our study provides the first evidence that CD8+ T lymphocytes are predominant in the inflammatory infiltrate. Further experiments aimed at assessing the target of the immune attack are needed. Analysis of the T cell repertoire could help determine if the T cell response is antigen-driven, possibly triggered by infectious or environmental agents or other antigen from injury to fascia or muscle.

REFERENCES