Bcl-2, p53 and c-myc expression in juvenile dermatomyositis

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Abstract

Objective

To investigate p53, bcl-2 and c-myc expression in muscle biopsies from children affected with juvenile dermatomyositis (JDM) and to verify a possible dysregulation of programmed cell death in this autoimmune disease.

Methods

Ten muscle biopsies of children affected with JDM were formalin fixed and paraffin embedded. After haematoxylin and eosin staining, immunohistochemistry was performed employing monoclonal antibodies, anti-p53, anti-bcl-2 and anti-myc. Two normal muscle biopsies were studied as controls.

Results

In the biopsies of JDM, two different patterns of myofibers damage were observed: the first, with zones characterised by necrosis; and the second, with zones where an apoptotic process was dominant. Immunoreactivity for bcl-2 was positive in 8 out of 10 biopsies. P53 and c-myc expression were not present in any case. No relationship between the degree of bcl-2 immunostaining and the disease course or outcome was observed.

Conclusions

The over-expression of bcl-2 protein in JDM may suggest a dysregulation of apoptosis in myofibers. Further studies are required in order to better understand the role of our data in the pathogenetic pathways of the disease.

Introduction

Juvenile dermatomyositis (JDM) is a multisystem autoimmune paediatric disease characterised by acute and chronic inflammation of striated muscles and skin (1). Its aetiology is still unknown, but the pathogenesis shows the involvement of both cell-mediated and humoral immunity. JDM is associated with increased polyclonal activation of B cells and autoantibody production (2). Apoptosis or programmed cell death differs from the process of necrosis from the biochemical and morphological points of view (3). Apoptosis is required for the normal development or function of cells, as seen during embryogenesis or in the amplification of immunogenic T-clones (4). Many details of the pathway leading to apoptosis have been clarified (4) and different kind of stimuli may induce apoptosis through genetically determined pathways (4). It has been suggested that autoimmune diseases and apoptosis are linked, in particular to cell activation and/or differentiation (5, 6).

Apoptosis is regulated by several protooncogenes and oncosuppressor genes that operate at different points along the pathways to programmed cell death (3). The proto-oncogene bcl-2 encodes a protein located in the inner membrane of mitochondria, and plays a regulatory role during the development and maintenance of adult tissues by the inhibition of apoptosis in specific cell types (7). In the immune system, the regulation of programmed cell death is important to ensure lymphocyte activation (8). The role of c-myc is of particular interest because of its bivalent function – it can trigger either cell proliferation or apoptosis. In vivo c-myc expression has been associated with a high turnover state in which both cell proliferation and apoptosis are present (9). The p53 protein is important in the initiation of apoptosis; it has been shown that it induces apoptosis in the presence of DNA damage caused by different agents (10). Moreover it has been shown a biochemical interaction between p53 and c-myc gene products (9). Dysregulated apoptosis seems to be linked to the development of autoimmune diseases (5, 6). In adult patients affected with systemic lupus erythematosus (SLE) (11, 12) and systemic sclerosis (13) a dysregulation of programmed cell death has already been shown. With regard to the expression of apoptotic gene products in paediatric autoimmune diseases, a study by our group has been recently reported in patients with juvenile onset SLE (14). JDM is characterised by the accumulation of CD4+ T cells and B lymphocytes in the perimisium and perifascicular area of the muscles (14). In myofibers the main lesion is necrosis as a result of microinfarcts and the number of capillaries is consistently reduced (15). The aim of the present study was to investigate p53, bcl-2 and c-myc expression in muscle biopsies from children affected with JDM in order to detect a possible dysregulation of apoptosis in this paediatric autoimmune disease.
Materials and methods
Formalin-fixed, paraffin-embedded muscle specimens from 10 children with JDM were retrospectively studied. All of the histological slides, stained with hematoxylin-eosin, were reviewed to confirm the original diagnosis. The study included 10 cases of JDM - 7 girls and 3 boys, aged 3 to 8 years (mean age 5 years 6 months) who fulfilled the Bohan and Peter (16) criteria for the diagnosis of definite JDM. In all patients the diagnosis of JDM was suspected within 1 to 4 months based on the first clinical manifestations (muscle weakness, heliotropic rash, Gottron’s sign) and abnormalities in the laboratory test results (creatine phosphokinase (CPK), aldolase, glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) increased levels). The diagnosis was confirmed in all cases by muscle biopsy (inflammatory infiltrates surrounding myofibers and vessels). All had active disease (17) and no steroids and/or immunosuppressive drugs had been administered before the biopsy was performed. The follow-up of the disease ranged from 1 year and 9 months to 5 years. The disease onset was acute in 6 out of 10 patients and subacute in the remaining 4.

After parental consent was obtained, biopsies were performed at admission in order to achieve a definite diagnosis of JDM. Two normal muscle biopsies, obtained from children undergoing bone surgery for traumatic lesions, were studied as controls.

Immunohistochemistry
Five-mu paraffin-embedded sections were mounted on xylene-coated slides and air dried. After dewaxing and blocking with endogenous peroxidase, sections were rinsed in water and then placed in 10 mM/L of citrate acid at pH 6.0. The sections were microwaved at 750W for 20 min, while making sure that they were covered with liquid the entire time. Following microwave heating, the sections were transferred to phosphate-buffered saline solution (PBS) and incubated in normal rabbit serum for 30 min to block non-specific interactions. Specimens were then treated with the following monoclonal antibodies overnight: p53, clone DO-7 (Dako, Denmark) which recognises both mutant and wild-type p53 proteins; bcl-2, clone 124 (Dako), and c-myc, clone 33 (Oncogene, Science, USA) at the dilution of 1:40.

Primary antibodies bound to antigen were detected using a standard streptavidin-biotine technique (LSAB kit, Dako) and visualised with diaminobenzidine. A light haematoxilin nuclear counterstaining was employed. The proportion of positive muscle cells was semiquantitatively scored as strong (+++), when 76% to 100% of cells were positive, moderate (++), when 50 to 75% of cells were positive and focal (+) when fewer than 50% of cells were positive. Negative controls were performed by replacing the primary antibodies with non-immune serum or PBS that resulted in negative staining.

Results
Histology
Microscopy showed focal inflammatory infiltrates (lymphocytes and macrophages) mainly surrounding myofibers and vessels. In some specimens the muscle bundles showed no significant degeneration; the myofibers were substantially well conserved and membrane integrity was maintained. In these cells, positive staining for bcl-2 protein was characterised as fine, brown cytoplasmatic granularity. (Fig. 1) The results of immunostaining are summarised in Table I. Bcl-2 was expressed in the majority of muscle specimens (8 out of 10 biopsies). Immunoreactivity for bcl-2 was confined to the cytoplasm of myofibers; no positive signals were detected in any of the other cell types present in the biopsies. Nuclear p53 and c-myc immunostaining was not detected in any specimen. The proportion of myofibers that stained for bcl-2 and the staining intensity was different in the examined biopsies (Table I).

Negative controls were carried out by replacing the primary antibodies with PBS. Moreover, two normal muscle biopsies were used as controls to compare with the cases of JDM studied. The immunoreactivity for the three proteins related to apoptosis control was negative.

Relationship between immunostaining results and clinical course
No correlation was found between the results of immunostaining for bcl-2 and either the course or the outcome of the
In fact, perifascicular myopathy represents a characteristic alteration of the disease and during the healing phase the multiple areas of focal necrosis are replaced by an interstitial proliferation of connective tissue.

Apoptosis is a physiological form of cell death that involves the activation of a series of proteins and is characterised by the maintenance of membrane integrity. In JDM myofibers, the over-expression of bcl-2 protein may suggest a dysregulation of programmed cell death. Bcl-2 proto-oncogene is involved in the oncogenesis of human follicular lymphoma and its product, a 26 Kd protein, prevents apoptosis. Expression of bcl-2 protein has mainly been detected in lymphoid tissue, although it has also been shown in several non-lymphoid fetal and adult tissues, some of which are characterised by a rapid cell turnover (e.g. epidermal and digestive epithelia). In fact, in self-renewing epithelia, the bcl-2 protein is expressed by stem cells and cells of the proliferative compartment.

Different hypotheses may be suggested to explain the increase of cytoplasmatic bcl-2 immunoreactivity in myofibers from JDM patients. As already hypothesised for p53 (13), the overexpression of bcl-2 might represent a secondary phenomenon due to the stress of myofibers involved in the chronic autoimmune process. On the other hand, bcl-2 immunoreactivity might represent a primary dysregulation of the control of programmed cell death in JDM myofibers, thus playing a key role in the disease pathogenesis. This event may be indirectly linked to the apoptotic factor, i.e. viruses, as possible triggering agents for JDM. In fact, an interaction between virus oncoproteins, i.e. LMP-1 encoded by Epstein Barr virus, and proto-oncogene products in the induction of cellular proliferation and tumor development has been demonstrated (20).

The lack of expression of p53 and c-myc confirms the functional interaction between these genes in the induction of apoptosis; thus, the cell death pathway in JDM seems to be activated mainly by bcl-2 proto-oncogene (21).

Bcl-2 immunostaining in our patients did not correlate with the disease course or disease outcome. In fact, the outcomes were different even with similar degrees of immunostaining. In our patients the muscle biopsies were performed at disease onset, before any therapy was introduced. It must be stressed that steroids, azathioprine, cyclophosphamide and methotrexate, the most widely employed therapies for autoimmune diseases, are potent inducers of apoptosis (5). Our patients were all without treatment. It would therefore be interesting to study bcl-2 expression in muscle specimens after such therapy, which was not possible in our children for ethical reasons. The evidence of modified bcl-2 protein immunoreactivity strongly suggests the possible role of dysregulated apoptosis in JDM and may indicate a pathway to be followed in designing new therapeutic approaches.

**References**


