SHORT REPORT

Immunohistological analysis of sarcoid myopathy

Dominique S Tews, Dieter E Pongratz

Abstract
In six cases of granulomatous myopathy immunohistological analysis showed a typical pattern with macrophages and T4 cells diffusely distributed throughout the cellular exudate. T8 lymphocytes were interspersed irregularly within the granulomatous cellular infiltrate early in granuloma maturation and in later stages predominantly confined to a lymphocytic mantle surrounding the granulomas. The cellular infiltrate displayed numerous activated HLA-DR and interleukin-2 receptor positive cells including cell proliferation. Increased connective tissue showed strong immunoreactivity for fibronectin and hyaluronate. Muscle fibres were negative for MHC class I molecules. Atrophic muscle fibres expressed desmin, a marker to distinguish desmin positive myogenic giant cells from desmin negative Langhans giant cells.

(J Neurol Neurosurg Psychiatry 1995;59:322–325)

Keywords: myopathy; granulomas; sarcoidosis

Generalised granulomatous inflammation of skeletal muscle with giant cell reaction may occur in infectious diseases, in collagenoses, in inflammatory bowel diseases, as a remote effect of neoplastic processes, in association with thymoma or myasthenia gravis, idiopathic especially in elderly woman, and as a typical feature of sarcoidosis.1–4 Although the aetiology of sarcoidosis is still unknown sarcoidosis is considered a disease of increased immunological activity.5

For organs other than muscle tissue, several publications describe the composition and formation of granulomatous lesions based on immunohistochemical analysis.6,7 Although there have been several reports about muscle involvement in sarcoidosis and Crohn’s disease an immunohistological analysis of the mononuclear cellular infiltrate and tissue in human skeletal muscle is still lacking.1–4

Materials and methods

MUSCLE TISSUE SPECIMENS

We studied limb muscle specimens from six patients with granulomatous lesions on routine histological stains. Five patients were subjected to biopsy for diagnosis of sarcoidosis. One patient was suspected of a myopathy in association with an inflammatory bowel disease. The biopsy specimens were frozen immediately in liquid nitrogen and stored at −80°C. Serial transverse sections of 5 μm thickness were cut at −25°C on a cryostat microtome and left overnight at room temperature.

IMMUNOHISTOCHEMICAL PROCEDURES

For immunohistochemical analysis we used the alkaline phosphatase antialkaline phosphatase (APAAP) technique. Monoclonal antibodies for CD19, CD4, CD8, macrophage, HLA-DR, HLA-ABC, interleukin-2 receptor, Ki67, desmin, vimentin, fibronectin, collagen IV (all Dakopatts), CD11, Leu-7, Leu-11b (all Becton Dickinson), and hyaluronate (Dr Schleicher, Darmstadt) were diluted with tris buffered saline (TBS) containing 10% fetal calf serum and incubated with the sections at room temperature for 30 minutes. Primary antibodies from rabbit required an additional antibody (monoclonal mouse antimouse Ig, Dakopatts), which enabled these rabbit primary antisera to react with the bridge antibody (rabbit antimouse immunoglobulin, Dakopatts) in the second step. The bridge antibody was connected with the APAAP enzyme complex (APAAP mouse monoclonal, Dakopatts) in the third step. Finally, the substrate was applied by the fast red method. After immunostaining the sections were stained with haematoxylin and mounted in glycerol-gelatin medium. In control sections, the primary antibody was either omitted or substituted with a non-specific respective Ig subclass.

IMMUNOHISTOCHEMICAL ANALYSIS

The complete analysis was done on 16 consecutive sections and in each section a defined area including an average of 500 muscle fibres was followed and analysed by

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Received 3 January 1995
and in final revised form
2 May 1995
Accepted 9 May 1995
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Distribution of mononuclear cells in granulomatous myopathy

<table>
<thead>
<tr>
<th>Cell type (%)</th>
<th>Perimysial and perivascular</th>
<th>Endomysial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages</td>
<td>51.3 (1.2)</td>
<td>39.2 (1.0)</td>
</tr>
<tr>
<td>Leu-7 + cells</td>
<td>0.0</td>
<td>0.8 (0.2)</td>
</tr>
<tr>
<td>Leu-11b + cells</td>
<td>0.0</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>B cells</td>
<td>1.9 (0.9)</td>
<td>3.2 (0.8)</td>
</tr>
<tr>
<td>T4 cells</td>
<td>31.6 (1.6)</td>
<td>44.6 (0.8)</td>
</tr>
<tr>
<td>T8 cells</td>
<td>15.0 (2.2)</td>
<td>13.1 (1.2)</td>
</tr>
<tr>
<td>T4/T8 ratio</td>
<td>2.1 (0.2)</td>
<td>3.6 (0.4)</td>
</tr>
<tr>
<td>HLA-DR + T cells</td>
<td>0.9 (0.9)</td>
<td>25.3 (2.5)</td>
</tr>
<tr>
<td>IL-2-R + T cells</td>
<td>2.1 (1.3)</td>
<td>4.5 (0.4)</td>
</tr>
<tr>
<td>CD11 + T cells</td>
<td>49.2 (5.9)</td>
<td>62.3 (2.8)</td>
</tr>
<tr>
<td>Ki67 + cells</td>
<td>0.7 (0.4)</td>
<td>3.6 (1.0)</td>
</tr>
</tbody>
</table>

All values are expressed as mean (SEM).

counting the positive cells. As some antibodies label more than one cell type, we did consecutive sections to estimate the number of the "relevant" cell type by calculating the difference between the total number of positive cells in one section and the number of positive, but non-relevant cells in the consecutive section: T4 lymphocytes = all CD4 + cells - (macrophages and monocytes); CD11 + T cells = all CD11 + cells - (Leu-7 + and Leu-11b + cells); HLA-DR + T cells = all HLA-DR + cells - (macrophages and B cells).

STATISTICAL ANALYSIS

All data were presented as means (SEM).

Results

On routine histological examination, numerous non-caseating granulomas consisting of Langhans giant cells, epitheloid histiocytic cells, and lymphocytes were present in connective tissue and within muscle bundles (figure A).

The table shows the distribution of the different cellular phenotypes. Within the granulomas macrophages were more frequent than lymphocytes, whereas lymphocytes dominated in the surrounding areas of the granulomatous lesions with lymphorrhages extending among adjacent muscle fibres. All granulomas contained numerous T4 positive cells distributed diffusely among the macrophages. Conversely, in most granulomas T8 positive cells were less numerous (figure B). In some granulomas T8 cells could be demonstrated throughout the granulomatous infiltrates (figure C) whereas in a second type of granulomata T8 cells were located as a rim around the granula but were absent in the centre (figure D). Most of these T8 lymphocytes were CD11 positive cells whereas T8 lymphocytes within the granuloma were often CD11 negative. Cytotoxic T cells invading non-necrotic muscle fibres were never seen. At all sites of muscle, T4 lymphocytes outnumbered T8 cells.

Leu-7 positive killer cells occurred in very small numbers only at endomysial sites. Likewise, activated and antigen presenting T cells bearing the HLA-DR antigen or the interleukin-2 (IL-2) receptor were found predominantly at endomysial sites. Macrophages also exhibited HLA-DR antigen reactivity. Throughout the granulomas and the perigranulomatous infiltrates, replicating Ki-67

(A) Well defined granuloma surrounded by normal appearing muscle fibres. Haematoxylin-eosin, calibration bar = 140 μm. (B) T8 cells spread within granulomatous tissue. Anti-T8, calibration bar = 53 μm. (C) Granuloma with T8 lymphocytes distributed diffusely within and around the granuloma. Anti-T8, calibration bar = 28 μm. (D) Sarcoid granuloma encircled by T8 lymphocytes. Anti-T8, calibration bar = 28 μm.
positive cells, predominantly lymphocytes, were scattered.

The increased connective tissue showed strong immunoreactivity for fibronectin and hyaluronate whereas muscle fibres displayed sarcolemmal labelling for collagen IV. Atrophic muscle fibres compressed by granulomas exhibited appreciable immunostaining for desmin. Likewise, myogenic giant cells reacted positively for desmin whereas multinucleated giant cells of the Langhans type did not react with desmin or with any other antibodies used. Neither normal appearing muscle fibres nor atrophic or degenerated fibres or myogenic giant cells showed immunoreaction for vimentin. Muscle fibres showed no expression of HLA-ABC.

Discussion
The immunophenotypic analysis of mononuclear cells in granulomas showed the typical distribution of cell subtypes described in granulomas for other organs. Granuloma formation is based on an inflammatory exudate. Several factors—that is, antigen mediated signals involving membrane binding—are discussed as initiating accumulation of inflammatory cells. Initially, numerous lymphocytes, predominantly T4 cells, are interspersed diffusely in the granulomatous infiltrate among macrophages and monocytes. These monocytes and macrophages have a high potential to differentiate into epithelioid and multinucleated giant cells representing maturation of the granuloma in which T8 cells are absent from central areas, but are confined to the periphery of the granulomas. Like other authors we suggest that these two histological types of granulomas, also found in our muscle samples, may represent different stages of granuloma maturation. Whereas T4 cells seem to be responsible for induction and modulation of granuloma development T8 cells are intended for maturation and focusing of granulomas in later stages. The predominance of CD11 positive cells at the border of the granulomas could be an attempt to prevent further spread of inflammatory infiltrates into surrounding areas.

Several unknown factors released by immunocompetent cells control immunological events of granuloma formation and maturation. IL-2, released by T4 cells, binds to IL-2 receptor bearing antigen activated T cells and selectively triggers their proliferation and differentiation into effector cells. As shown in other studies, we also found in our granuloma numerous activated HLA-DR positive as well as IL-2 receptor bearing T cells. Besides cellular redistribution and migration from the bloodstream, a second major mechanism for cellular accumulation entails cell proliferation at the sites of disease activity indicated by numerous Ki-67 positive lymphocytes and macrophages.

The role of several active mediators including IL-2, collagen, and fibronectin in the development of fibrosis has not been clarified. Fibronectin is regarded as a competent factor in the early stage of fibroblast migration and proliferation which stimulates fibroblast replication. We found an enhanced expression of fibronectin as well as of hyaluronate within and around granulomas together with an increase of connective tissue. Increases in both extracellular matrix components are also described for bronchoalveolar lavage fluid in sarcoidosis. Although the destruction of muscle fibres seems to be mainly secondary by derangement and compressive destruction there is evidence that monocytes and macrophages are able to release a type IV collagenase which may impair the muscle plasma membrane.

Atrophic and compressed muscle fibres displayed strong expression of desmin and it is under debate if this is a sign of fibre breakdown or of regeneration. Desmin, however, seems to be a useful marker to distinguish myogenic giant cells and multi-nucleated giant cells of the Langhans type. Myogenic giant cells can be positively identified by their expression of desmin absent in Langhans type giant cells.

At the present time, we are not able to view our findings from an immunohistolical analysis as specific for sarcoidosis. Perhaps, more specific markers will be of diagnostic help. Nevertheless, there are some features specific for granulomatous myopathy and of diagnostic use for differential diagnosis in inflammatory myopathies. Besides the typical formation of T8 lymphocytes in granulomatous infiltration, no other inflammatory myopathy shows such a high percentage of T4 cells and T4/T8 ratio. Conversely, both polymyositis and inclusion body myositis show clear dominance of T8 lymphocytes invading non-necrotic muscle fibres.

Dermatomyositis displays, likewise, a high amount of T4 cells but also a distinct increase in B cells both predominantly located at perimysial sites. Other inflammatory myopathies show HLA-ABC expression of muscle fibres, which is lacking in granulomatous myopathy.

This study, supported by the Friedrich-Baur-Stiftung, was part of a thesis submitted to the Ludwig-Maximilians-University, Munich, Germany, 1992, and was presented at the 9th meeting of the Scientific Council of the German Society for the Treatment of Muscle Diseases, Hamburg, Germany, September 1989. We are grateful to Dr Schleicher for providing the antibody against hyaluronate and thank Eva Wiens for routine histological preparations, Walther Meffert for photography, and Astrid Woiber for linguistic assistance.

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Haslam PL, Parker DJ, Townsend PJ. Increases in HLA-DQ, DP, DR, and transferrin receptors on alveolar macrophages in sarcoidosis and allergic alveolitis compared with fibrosing alveolitis. Chest 1990;97:651-61.


Announcement from the British Neuropyschiatry Association: 1996 summer meeting

The 1996 Summer meeting will be held on 14-16 July at Robinson College, Cambridge. It will include topics on neurodevelopment, language, and the presentation of short scientific papers and single case videos by members. The Association's AGM will be held on 16 July.

For further details of these meetings please contact: Sue Farratt, Administrative Assistant, BNPA, 17 Clocktower Mews, London N1 7BB. Telephone/Fax: 0171 226 5949.

For details of membership of the BNPA, which is open to medical practitioners in psychiatry, neurology, and related clinical neurosciences, please contact: Dr Jonathan Bird, Secretary BNPA, Burden Neurological Hospital, Stoke Lane, Stapleton, Bristol, BS16 1QT. Telephone: 01179 701212 ext 2925/2929 or Sue Farratt at the address given above.

CORRECTION


Dr CC Tijssen should be included as an author for this case conference, which should read: Harrison MJG, Teepen JLM, Tijssen CC, A case of recurrent headache and neurological deficit. J Neurol Neurosurg Psychiatry 1995;59:322-7.

BOOK REVIEWS

All titles reviewed here are available from the BMJ Bookshop, PO Box 295, London WC1H 9TE. Prices include postage in the United Kingdom and for members of the British Forces Overseas, but overseas customers should add £2 per item for postage and packing. Payment can be made by cheque in sterling drawn on a United Kingdom bank, or by credit card (Mastercard, Visa or American Express) stating card number, expiry date, and your full name.


This is a highly impressive and well researched text book covering every aspect of modern neurovascular surgery. The editors have assembled 125 contributors who are all well respected in their fields. As a result, the text book is extensive with global reference. The book is divided into six main parts consisting of general principles, occlusive disease, haemorrhagic conditions, vascular compression, spinal vascular disease, and vascular injuries. All sections are well covered and include essential medical neuroanaesthetic and interventional aspects as well as the direct surgical descriptions. Despite multiauthorship, the editors appear to have kept overlap down to an absolute minimum.

What I found particularly impressive was the detail in which the key trials and clinical papers have been documented. As a result, I have found the book useful in extracting key material and figures with ease. The extensive coverage makes this book suitable for interested parties other than surgeons. I just wonder whether it may have been more appropriately entitled "The Treatment of Neurovascular Conditions" so as not to deter the non-surgical community. I would personally recommend this book to neuro-intensivists and neuro-radiologists in addition to neurosurgeons of all grades.

In summary, this really is an excellent book which has been put together by highly respected workers in the discipline of neurovascular conditions. I congratulate them on what must have been an enormous effort.
PETER KIRKPATRICK


This book, inspired by the fortuitous discovery of MPTP induced parkinsonism in a small group of Californian drug addicts, is a joy to read. It represents an attempt to review and explore all the theories that have been put forward to account for the cause of Parkinson's disease (PD); a review that extends to a bibliography of 2413 references! In addition to presenting the possible theories, it openly criticises and highlights the shortcomings of studies set up to unravel the aetiology of this common yet incurable disease.

The book opens with an excellent discussion on the causes of parkinsonism and how these relate to idiopathic PD (IPD). The discussion then goes into discussing and the difficulties in identifying IPD from other forms of parkinsonism, especially other neurodegenerative conditions such as multisystem atrophy. The difficulty in establishing and verifying a diagnosis of IPD antemortem seems to have been helped by the advent of functional imaging with the PET scanner. Although this technique is not widely available, problems are already appearing on the horizon as pointed out by Golbe in his chapter on the genetics of PD. He makes the point that some twin studies have shown abnormal fluorodopa PET scans similar to those in PD; in twins who do not have PD but only a postural tremor; a point further discussed in later chapters on the neuroepidemiology and comorbidity in PD.

This initial discussion on what constitutes PD is fundamental to understanding what may cause it. However, in addition the pathology of the condition has to be explained and Forno gives an excellent account on the neuropathology of PD. This chapter makes another important point—namely, that although the dopaminergic nigral neurons bear the brunt of the pathology—they are not the only neurons to be involved in the disease process. This point must therefore be taken into account when any theory purporting to explain the aetiology of this condition is put forward.

The diagnosis and pathology of IPD having been established the question then arises as to what causes the neurons to be lost. Irwin and Langston begin by presenting possible mechanisms of cell death, although no discussion on the ontogeny of the nigrostriatal pathway is given. This is a shame as it may be relevant to the mechanism of cell death in the disease state. Nevertheless, the possible cellular mechanisms that cause the dopaminergic neurons to be lost is then taken up in later chapters that specifically address the possibilities of endogenous and exogenous toxins as aetiological agents, including a discussion of MPTP itself. This discussion on toxins raises the further question as to whether levodopa itself is toxic to the nigral dopaminergic neuron and so catalyses the disease process. A significant amount of in vitro work is omitted from the discussion but the overwhelming work from those studies agrees with the conclusion put forward in this book—namely, that although this is a theoretical risk there is no convincing evidence that it is a dominant factor driving the pathogenesis of this condition.

The only criticisms I have of this book are minor ones, in that some of the chapters read rather too much like lists (for example, chapter 7) whilst others are unnecessarily technical (for example, chapter 11 on assessment of predictors). Overall, though, this book is good fun. It presents a large amount of information in an interesting and critical way and I would therefore strongly recommend it to all who wonder at the cause of this major neurodegenerative disorder.

ROGER BARKER