Neuropathology of CNS disease in Langerhans cell histiocytosis

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Summary
CNS involvement in Langerhans cell histiocytosis (LCH) is a rare but potentially devastating disorder. Different types of involvement have been described by MRI. CNS changes can have space-occupying or degenerative character. Little is known about the underlying neuropathology and pathophysiology. In our study we reviewed brain samples from 12 patients with LCH. The neuropathology findings were correlated with the MR morphology and the clinical presentation. By neuropathology, three types of lesions were distinguished. (i) Circumscribed granulomas within the brain’s connective tissue space corresponded to tumorous lesions in the meninges or choroid plexus on MRI. They showed a composition similar to Langerhans granulomas in peripheral organs, with variable presence of CD1a-reactive cells and pronounced CD8-positive (+) T-cell infiltration. (ii) Granulomas occur within the brain’s connective tissue spaces with partial infiltration of the surrounding CNS parenchyma by CD1a-reactive histiocytes. This was associated with profound T-cell-dominated inflammation and severe neurodegeneration, characterized by a nearly complete loss of neurons and axons, and gliosis. (iii) Neurodegenerative lesions lacking infiltration of CD1a+ cells, mainly affecting the cerebellum and brainstem, exhibited a profound inflammatory process dominated by CD8-reactive lymphocytes, associated with tissue degeneration, microglial activation and gliosis. Patients with such lesions showed different stages of neurological deterioration. This study indicates that neurodegeneration in LCH occurs on the background of a T-cell-dominated inflammatory process and is characterized by neuronal and axonal destruction with secondary demyelination, resembling paraneoplastic encephalitis.

Keywords: demyelinisation; Langerhans cell histiocytosis; neurodegeneration; paraneoplastic encephalitis

Abbreviations: LCH = Langerhans cell histiocytosis


Introduction
Langerhans cell histiocytosis (LCH) is a rare disease of the dendritic cell system that may affect almost any organ. Recent studies indicate that LCH is caused by an uncontrolled clonal proliferation of dendritic cells with Langerhans cell characteristics. An abnormal interaction of LCH cells with T cells, with high cytokine levels, has been demonstrated (Egeler and D’Angio, 1995; Arceci, 1999; Egeler et al., 1999). Chemokines and chemokine receptor profiles suggest that LCH features the characteristics of chronic inflammation (Laman et al., 2003). Typical granulomas consist of an accumulation of CD1a-positive (+) Langerhans cells, indeterminate and interdigitating cells, macrophages and T-lymphocytes, and variable numbers of multinucleated giant cells and eosinophils. The peculiar lesional morphology is influenced by the site and age of the lesion (Nezelof, 1979; Schmitz and Favara, 1998).

CNS involvement in LCH has been recognized since the early reports of the disease (Schueller, 1915; Christian, 1920; Hand, 1921). During the past decade, a variety of CNS lesions has been diagnosed by MRI in an increasing number of LCH patients (Barthez et al., 2000; Prayer et al., 2004).
The most common CNS manifestation in LCH is infiltration of the hypothalamic pituitary region by LCH granuloma, frequently leading to diabetes insipidus and anterior pituitary hormone deficiency. Neurodegenerative changes, the second most frequent pattern, comprise mostly bilateral symmetric lesions in the cerebellum and basal ganglia of variable signal quality on MRI, depending on site and stage of the lesion. Less frequently lesions in the extraaxial spaces, i.e. the meninges, pineal gland and choroid plexus, are observed (Prayer et al., 2004).

Neuropathology investigations have been reported in anecdotal cases (Feigin, 1956; Kristensson, 1966; Yamaguchi et al., 1972; Kepes, 1979; Poe et al., 1994). A comprehensive treatise on the disorder based on conventional neuropathology of autopsy material was provided by Kepes (1979). In 1994, a review on LCH CNS patients was published by Grois and colleagues, introducing a classification scheme of the MR or computed tomography changes based on 23 patients (Grois et al., 1994). Haematoxylin–eosin-stained slides from brain biopsy samples were available for 13 patients, but only few immunocytochemical studies could be performed on the scarce available tissue. Over the past decade our group has continued to study the phenomenon of CNS LCH. In 2004, we needed to adapt our previous classification scheme according to new insights gained by reviewing MRIs from 163 patients (Prayer et al., 2004). In an attempt to correlate the current conception with neuropathological findings, we performed this cooperative study, evaluating brain biopsy or autopsy material from 12 LCH patients by applying modern immunocytochemical techniques.

Patients and methods

Patients

Clinical data from 12 patients with neuropathology studies were collected at the LCH Study Reference Center. Ten of the patients were males and two females. Their ages at diagnosis of LCH ranged from 18 months to 28 years (median 3 years). The diagnosis of LCH was established from peripheral biopsies in nine patients and from CNS biopsies in three patients, according to the criteria of the Histioocyte Society (Broadbent et al., 1989).

MRI

From each of the 12 patients, the brain MRI study closest to the time of biopsy was reviewed by an experienced neuroradiologist (D.P.). Lesions on MRI were classified by anatomical regions and signal quality as described by Prayer et al. (2004). Neurodegenerative changes were defined as hyperintensities on T1-weighted images in the cerebellar dentate nucleus, with hypo- or hyperintensity on T2-weighted images and possible extension to the perinuclear white matter, and/or T1-weighted hyperintensity of the basal ganglia (Prayer et al., 2004).

Neuropathology

Material from 11 brain biopsies and one autopsy specimen was available for this study. The material was routinely fixed in formalin and embedded in paraffin. Serial sections were cut and stained with haematoxylin–eosin, Luxol Fast Blue for myelin and Bielschowsky silver impregnation for axons. Haemosiderin was detected with the Prussian Blue reaction. Immunocytochemistry was performed with antibodies against: CD3 (Dakopatts, Hamburg, Germany), CD8 (Labvision, Freemont, CA, USA), Granzyme B (Labvision), CD20 (NeoMarkers, Freemont, CA, USA), CD68 (Dakopatts), CD1a (Novocastra, Newcastle, UK), MHC class I (β2 microglobulin; Dakopatts), MHC class II (HLA-D; Dakopatts), S-100 (NeoMarkers), glial fibrillary acidic protein (GFAP; Dakopatts), neurofilament (Chemicon, Temecola, CA, USA), β-amyloid precursor protein (Boehringer, Mannheim, Germany) and cyclic nucleotide phosphodiesterase (Sternberger Monoclonals, Lutherville, MD, USA). Immunocytochemistry was performed with a biotin–avidin technique (Bien et al., 2000). Direct quantitation of CD4+ T-lymphocytes is problematic in human archival autopsy and biopsy material due to limited staining in formalin-fixed and paraffin-embedded tissue and the variable expression of CD4 antigen on activated microglia. Therefore, cells staining positive for CD3 but negative for CD8 were classified as CD4+ T-cells.

Results

Clinical presentation and course (Table 1)

Upon diagnosis, six patients presented with multisystem disease and six patients had single system involvement. Two patients had isolated CNS involvement. Eleven patients received LCH directed systemic therapy including prednisone, vinblastine, etoposide, methotrexate, cytarabino- side, vincristine or cyclosporin, and biphosphonates prior to the CNS biopsy. Patient 10 received irradiation of the skull with 10 Gy. In patient 4, a tumour in the hypothalamic pituitary region was surgically removed.

Eight patients presented with diabetes insipidus 11 months prior to the diagnosis of LCH, to up to 6 years thereafter. In seven of these anterior pituitary hormone deficiencies were also reported. Nine patients were reported to have developed neurological symptoms comprising ataxia leading to inability to walk, dysarthria, cognitive and memory deficits, and severe headaches.

CNS biopsy

In four patients biopsy was performed to yield the diagnosis of a CNS mass lesion upon initial presentation. In seven patients a biopsy was obtained to clarify the nature of parenchymal CNS changes up to 13 years after initial diagnosis of LCH. Autopsy in patient 12 was done 22 years after diagnosis.

MRI findings at the time of biopsy (Table 2)

Changes in the hypothalamic pituitary region were seen in eight patients. Meningeal masses were found in three patients and choroid plexus tumours in two. T2-weighted images from 10 patients showed dilated Virchow Robin spaces, and in two patients no T2 images were available.
Six patients exhibited intraparenchymal signal changes in the cerebellum, seven patients in the basal ganglia and two patients in the pons. Four patients had pontine lesions with a vascular enhancement. In patient 11 a supratentorial periventricular leukencephalopathy-like pattern was observed, with poorly defined patchy areas of hypointensity on T1-weighted images or hyperintensity on T2-weighted images without contrast enhancement. Atrophy was noted in five patients.

**Neuropathology**

Neuropathological review of biopsy and autopsy specimens revealed three different types of lesions: (i) circumscribed granulomas within the brain’s connective tissue space, such as the meninges or choroid plexus (Fig. 1); (ii) granulomas within the brain’s connective tissue spaces with partial infiltration of the surrounding CNS parenchyma (Fig. 2); and (iii) neurodegenerative lesions, mainly affecting the cerebellum and the brainstem (Fig. 3).

Circumscribed granulomas in brain connective tissue spaces formed large space-occupying lesions in the extraxial spaces, such as the meninges (patient 2) and the choroid plexus (patient 1), or were found as multiple small nodules in the meninges (patient 7). The lesions were sharply demarcated from the CNS tissue and consisted of a mixture of histiocytes, foamy macrophages, multinucleated giant cells, lymphocytes, plasma cells and eosinophils. The ratio of T-cells, B-cells, plasma cells and granulocytes varied among the different granulomas. Their cellular composition was similar to Langerhans granulomas in peripheral organs, although in CNS granulomas CD8+ T-cells generally outnumbered CD3+CD8− T-cells.

The presence and number of CD1a+ cells was variable, while dominating in some lesions, they were scarce or even absent in others, partly reflecting the stage of granuloma

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**Table 1 Clinical presentation and course**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age*</th>
<th>Sex</th>
<th>Organs involved*</th>
<th>Neurological symptoms at biopsy</th>
<th>Course observation (time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 years</td>
<td>F</td>
<td>Bone-skull, liver, spleen, lungs, hematopoietic system</td>
<td>Headaches</td>
<td>NAD, stable NS, cognitive and memory deficits (14 years)</td>
</tr>
<tr>
<td>2</td>
<td>2 years 2 months</td>
<td>M</td>
<td>Bone-skull</td>
<td>No symptoms</td>
<td>Three reactivations: skull, ataxia (7 years 8 months)</td>
</tr>
<tr>
<td>3</td>
<td>8 years 3 months</td>
<td>M</td>
<td>HPR (DI)</td>
<td>No symptoms</td>
<td>Multiple reactivations (3 years 9 months)</td>
</tr>
<tr>
<td>4</td>
<td>13 years</td>
<td>M</td>
<td>Bone-skull base, jaw, mastoids, lungs, HPR (DI, TSH, ACTH)</td>
<td>No symptoms</td>
<td>One bone reactivation (3 years)</td>
</tr>
<tr>
<td>5</td>
<td>1 year 9 months</td>
<td>F</td>
<td>Bone-mastoids, bone marrow, HPR (DI, GH)</td>
<td>Headaches</td>
<td>Three reactivations: HPR, lymph node, mastoids cognitive and memory deficits (13 years)</td>
</tr>
<tr>
<td>6</td>
<td>28 years</td>
<td>M</td>
<td>Cerebellar tumour, HPR mass (DI, hypogonadism), skin, bone-mastoid</td>
<td>Ataxia, fine motor deficits, dysarthria</td>
<td>NAD, NS better (4 years)</td>
</tr>
<tr>
<td>7</td>
<td>1 year 6 months</td>
<td>M</td>
<td>Bone-jaw</td>
<td>Cognitive deficits</td>
<td>One reactivation: skull, HPR, progressive NS: ataxia, dysarthria, dysmetria dysphagia (16 years 6 months)</td>
</tr>
<tr>
<td>8</td>
<td>3 years 5 months</td>
<td>M</td>
<td>Bone-skull, jaw, skin, HPR (DI)</td>
<td>Ataxia, cognitive deficits</td>
<td>NAD, progressive NS (12 years)</td>
</tr>
<tr>
<td>9</td>
<td>15 years</td>
<td>M</td>
<td>Pineal gland tumour</td>
<td>Ataxia, cognitive and memory deficits, dysfunctional voiding</td>
<td>NAD, progressive NS: tetraparesis, dysphagia, dysarthria (7 years)</td>
</tr>
<tr>
<td>10</td>
<td>4 years</td>
<td>M</td>
<td>Bone-orbits</td>
<td>Tetraparesis, cognitive and memory deficits, dysarthria, dysphagia, nystagmus</td>
<td>One reactivation: lung, progressive NS (25 years)</td>
</tr>
<tr>
<td>11</td>
<td>2 years 5 months</td>
<td>M</td>
<td>Bone-skull, mastoids, HPR (DI, GH)</td>
<td>Ataxia, behavioural disturbance</td>
<td>NAD, progressive NS: tetraparesis, dysarthria (16 years)</td>
</tr>
<tr>
<td>12</td>
<td>1 year 11 months</td>
<td>M</td>
<td>Bone-skull, mastoid</td>
<td>Tetraparesis, dysarthria</td>
<td>Died age 24 years, NAD, progressive NS (22 years)</td>
</tr>
</tbody>
</table>

*At diagnosis. M = male; F = female; HPR = hypothalamic pituitary region; DI = diabetes insipidus; GH = growth hormone deficiency; ACTH = adreno-corticotrophic hormone deficiency; TSH = thyroid-stimulating hormone deficiency; NAD = non-active LCH disease (outside CNS); NS = neurological symptoms.
<table>
<thead>
<tr>
<th>Patient</th>
<th>MRI at time of neuropathology</th>
<th>Site/mode of CNS biopsy (time from LCH diagnosis)</th>
<th>Neuropathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calcified choroid plexus mass, dilated VRS</td>
<td>Choroid plexus$^a$ (+1 year 6 months)</td>
<td>CD1a+ granuloma</td>
</tr>
<tr>
<td>2</td>
<td>Epidural masses, dilated VRS</td>
<td>Meningeal tumour$^c$ (+5 years 6 months)</td>
<td>CD1a+xanthomatous granuloma</td>
</tr>
<tr>
<td>3</td>
<td>Hypothalamic tumour, missing 'bright spot', dilated VRS, ND in cerebellum and basal ganglia</td>
<td>HPR$^a$ (at diagnosis)</td>
<td>CD1a+ granuloma with infiltration of surrounding CNS parenchyma</td>
</tr>
<tr>
<td>4</td>
<td>Hypothalamic tumour, missing 'bright spot', dilated VRS</td>
<td>HPR$^a$ (at diagnosis)</td>
<td>CD1a++ granuloma with infiltration of surrounding CNS parenchyma</td>
</tr>
<tr>
<td>5</td>
<td>Hypothalamic tumour, missing 'bright spot', enhancing vascular lesions in pons, dilated VRS, ND in cerebellum and basal ganglia + Fe deposit</td>
<td>HPR$^a$ (+7 years 7 months)</td>
<td>CD1a+ granuloma with infiltration of surrounding CNS parenchyma</td>
</tr>
<tr>
<td>6</td>
<td>Infundibular thickening, missing 'bright spot', epidural, cerebellar mass, enhancing vascular lesions in pons, dilated VRS, ND in basal ganglia</td>
<td>Cerebellar tumour$^c$ (at diagnosis)</td>
<td>CD1a++ granuloma</td>
</tr>
<tr>
<td>7</td>
<td>Infundibular thickening, missing 'bright spot', enhancing vascular lesions in pons and cerebellum, cerebellar meninges, dilated VRS</td>
<td>Cerebellum$^c$ (+4 years 9 months)</td>
<td>Cerebellar meninges: CD1a– granulomatous inflammation; cerebellar cortex and subcortical WM: massive CD8+ T-cell infiltration, microglia activation* and neurodegeneration</td>
</tr>
<tr>
<td>8</td>
<td>Thread-like infundibulum, missing 'bright spot', dilated VRS, ND in cerebellum, pons and basal ganglia, cerebellar atrophy</td>
<td>Cerebellar surface$^c$ (+2 years 2 months)</td>
<td>Low infiltration of tissue with CD8+ T-cells and neurodegeneration</td>
</tr>
<tr>
<td>9</td>
<td>Enhancing vascular lesions in pons, dilated VRS, ND in basal ganglia</td>
<td>Pineal gland$^a$ (at diagnosis)$^1$, cerebellum$^c$ (+8 months)</td>
<td>(CD1a– xanthomatous granuloma)$^1$: massive CD8+ T-cell infiltration, microglia activation*, neurodegeneration; enlarged VRS filled with CD68+ cells</td>
</tr>
<tr>
<td>10</td>
<td>ND in cerebellum, cerebellar atrophy</td>
<td>Cerebellum$^d$ (+13 years)</td>
<td>Leukoencephalopathy with reactive gliosis, microglia activation*</td>
</tr>
<tr>
<td>11</td>
<td>Thread-like infundibulum, missing 'bright spot', ND in cerebellum and basal ganglia, supra- and infratentorial leukoencephalopathy</td>
<td>Periventricular white matter$^c$ (+2 years 7 months)</td>
<td>Leukoencephalopathy with reactive gliosis, little CD8+ T-cell infiltration, microglia activation*</td>
</tr>
<tr>
<td>12</td>
<td>Thickened pituitary stalk, missing 'bright spot', choroid plexus mass, dilated VRS, ND in cerebellum, pons and basal ganglia + iron deposit, midbrain atrophy</td>
<td>Autopsy (+22 years)</td>
<td>Diffuse T-cell-mediated encephalitic process of the entire brain with inflammation associated tissue destruction predominantly in the cerebellum and the basal ganglia</td>
</tr>
</tbody>
</table>

*Defined by expression of MHC molecules and CD68+; CD1a+ = some CD1a-positive cells; CD1a++, abundant CD1a-positive cells. According to pathology report (tissue from the first biopsy not available for review). HPR=hypothalamic pituitary region; ND = neurodegenerative disease; VRS = Virchow Robin spaces; WM = white matter; $^c$ = open biopsy.
formation. In ‘younger’ lesions, Langerhans histiocytes with the dendritic cell marker CD1a on their surface were demonstrated (patients 1 and 6). In patient 2 a biopsy from a meningeal tumour, performed >5 years after initial diagnosis, revealed a xanthogranuloma, nearly exclusively composed of foamy CD68+ macrophages, with no CD1a+ cells detectable.

Granulomas with partial infiltration of the surrounding CNS parenchyma were found in a biopsy of a cerebellar tumour (patient 6) and in biopsies from infundibular tumours invading the adjacent hypothalamus (patients 3, 4 and 5). The lesional core was essentially similar to that described above for meningeal granulomas. However, the adjacent CNS tissue
was diffusely infiltrated by CD1a+ histiocytes, CD68+ macrophages and T- and B-cells, while granulocytes and eosinophils were scarce. This diffuse cellular infiltration was associated with a nearly complete loss of neurons and axons. In areas of dense granulomatous infiltration astrocytes were reduced, but formed a dense glial scar in the periphery of the lesion. The zone of granulomatous infiltration of the tissue was surrounded by a much larger area of inflammation, without CD1a+ or S-100+ histiocytes or foamy macrophages. Inflammatory cells in this region were mainly composed of CD8+ T-lymphocytes, while other T-cells, B-cells or plasma cells were rare. This T-cell dominated inflammatory process concurred with profound activation of microglia and the expression of class I and II MHC molecules, as well as partial loss of neurons and oligodendrocytes and profound astrogliosis.

**Neurodegenerative lesions**

The full spectrum of neurodegeneration was observed in the autopsy of patient 12. There was a diffuse inflammatory process affecting the whole brain, dominated by CD8+ lymphocytes, which were found in perivascular inflammatory cuffs as well as diffusely dispersed throughout the tissue. Many of these cells contained Granzyme B-reactive cytoplasmic granules as a sign of cytotoxic activation. Rare CD20+ B-cells
Fig. 3 Neurodegenerative lesions in patient 12. (A) Symmetric hypointensities within the pallidum, compatible with pathological hemosiderin or ferritin deposition on a T2-weighted image. (B) Corresponding axial T1-weighted image. Symmetric areas of high signal intensity within the pallidum. (C) Axial T2-weighted image. Symmetric high intensity signals in the dentate nuclei and surrounding white matter. (D) Cerebellar cortex and white matter with cortical atrophy and massive degeneration in the deep white matter. Asterisk in D, E and K marks the area with most active inflammation and tissue damage. Luxol Fast Blue myelin stain. Magnification ×2. (E) Bielschowsky silver impregnation of the adjacent section shows profound axonal loss in the deep white matter lesions. Magnification ×2. (F–I) Perivascular inflammatory infiltrates in the white matter, showing accumulation of CD3+ (F) and CD8+ (G) T-lymphocytes and CD68+ macrophages (H), but no CD1a+ histiocytes (I). Magnification ×50. (J) CD8+ T-cells are not only present around vessels, but also diffusely infiltrate the CNS tissue. Magnification ×100. (K) The massive inflammatory reaction is associated with profound expression of MHC class I molecules, which is especially profound in the area of active tissue damage (*). Magnification ×2. (L) Old lesion in the deep white matter, showing massive reduction of axonal density and extensive widening of the perivascular space. Bielschowsky silver impregnation. Magnification ×50. (M) Massive loss of Purkinje cells in the cerebellar cortex is a consistent feature of chronic neurodegenerative LCH lesions. In this section only a single Purkinje cell is left over; axonal spheroids can be seen in the granular layer, suggesting acute and ongoing axonal injury in the residual neurons. Immunocytochemistry for phosphorylated neurofilament. Magnification ×80. (N) Neuron in the cerebellar dentate nucleus with eccentric nucleus and accumulation of filamentous material in the cytoplasm, suggesting retrograde degeneration. Bielschowsky silver impregnation. Magnification ×400. (O) In the basal ganglia massive deposition of hemosiderin is found in macrophages. Prussian Blue reaction. Magnification ×150.
were found only in the perivascular position. This T-cell-mediated inflammatory reaction concurred with profound microglia activation and tissue degeneration. Activated microglia expressed class I and class II MHC molecules and the phagocytic activation marker CD68. In none of the tissue blocks were focal granulomas present. No CD1a+ cells were found. Tissue degeneration was most pronounced in the cerebellum, the brainstem including pons and mesencephalon, the infundibulum including the optic nerve and chiasm, and the basal ganglia. Solely in the basal ganglia, abundant perivascular haemosiderin-loaded macrophages were detected. The neurodegenerative changes were most severe in the cerebellum. The deep cerebellar white matter was atrophic, showing a profound loss of axons and myelin with reactive glial scar formation. There was no primary segmental demyelination and no preferential loss of oligodendrocytes. The Virchow Robin spaces around large vessels were massively enlarged and contained some T-cells and macrophages. Atrophy was found also in the cerebellar cortex, reflected by a nearly complete loss of Purkinje cells and a reduction of neurons in the granular layer. Severe neuronal loss and dystrophic neurons were also seen in the cerebellar nuclei. Parenchymal lesions in other brain areas were similar to those in the cerebellum, but less extensive.

Active ongoing tissue injury and destruction was detected in some of the lesions with more pronounced inflammatory reaction. Many of the CD8+ lymphocytes contained cytotoxic, Granzyme B-reactive granules. MHC class I expression was encountered in active lesions not only on inflammatory cells and microglia, but also on some astrocytes and neurons. In these areas, many axons exhibited focally accumulated amyloid precursor protein as a reaction to impaired axonal transport and acute axonal degeneration. This was associated with secondary destruction of myelin in affected nerve fibres. The pathological alterations in the cerebellum in patient 7 were similar to those described above, but the degeneration of the cerebellar tissue was less advanced. However, in contrast to patient 12, small LCH granulomas, without CD1a+ histiocytes, were apparent in the meninges.

In two small samples from stereotactic biopsies of the deep white matter (patients 10 and 11), merely leukencephalopathy with reactive gliosis and microglial activation was detected.

Discussion

This study highlights new aspects of the neuropathology of CNS disease in LCH.

On brain MRI of LCH patients, several distinct lesion types have been described (Prayer et al., 2004). Sharply demarcated tumour-like lesions can be seen in the meninges or the choroid plexus. Neuropathologically, such lesions present as typical granulomas with CD1a+ LCH cells or as xanthogranulomas. The morphology of LCH lesions may change depending on their stage (Nezelof, 1979). One may speculate that xanthomatous lesions present ‘burned-out’ lesions that have lost the characteristic LCH morphology. On the other hand, this particular phenotype could be determined by the local environment. In the brain, the differential diagnosis of xanthomatous LCH granuloma and juvenile xanthogranuloma may be challenging, and sometime diagnostic proof remains dependent on extracerebral lesions (Freyer et al., 1996).

In contrast to mass lesions, smaller, sharply demarcated punctate lesions within affected brain tissue can also be observed on MRI (Fig. 1i). No biopsy was available from such lesions in the pons in patient 7, but they may represent small granulomas in the meninges or the Virchow Robin spaces, surrounding large arteries and veins. Furthermore, in all but two patients of this series, hyperintensities on T2-weighted images following a vascular pattern were observed in the parenchyma, apparently corresponding to distended Virchow Robin spaces. Whether these distended Virchow Robin spaces present perivascular granulomas or the residuum of cleared granulomas remains to be determined.

In addition to focal lesions, diffuse alterations of the brain parenchyma have been observed on MRI, and have been interpreted as neurodegenerative changes. These lesions are most severe in the cerebellar dentate nuclei and deep cerebellar white matter, but may also affect the brainstem, basal ganglia and sometimes the forebrain hemispheres. The present study shows that these lesions reflect a diffuse inflammatory process within the CNS parenchyma, associated with neuronal and axonal degeneration, and secondary myelin loss. The massive neuronal and axonal loss in the cerebellum results in atrophy of the cerebellar cortex and white matter.

Kepes distinguished four different CNS pathology patterns: lesions extending to the CNS from neighbouring bones, meningeal granulomas, intraparenchymal lesions, which may either be circumscribed or diffuse, and a combination of the above manifestations in a single patient (Kepes, 1979). This classification is similar to that proposed in this study for describing the LCH granulomas in the brain, but we expanded this classification by defining the inflammatory nature of the ‘neurodegenerative’ lesions.

Kepes pointed out that histiocytosis is essentially a proliferative disease of mesenchymal tissue and thus can arise in the brain, where mesenchymal structures, like meninges or choroid plexus, are present. However, he also noted that such lesions sometimes may penetrate the superficial or perivascular glia limitans and infiltrate the CNS parenchyma. This is consistent with more recent experimental data demonstrating that dendritic cells are present in the connective tissue spaces of the normal brain, but not in the parenchyma (McMenamin, 1999; Pashenkov et al., 2003). Under pathological conditions, such as ischaemia or inflammation, however, dendritic cells can be recruited into parenchymal areas (Serafini et al., 2000; Kostulas et al., 2002). These cells seem mainly to be recruited from the peripheral immune system, but may also in part differentiate from local microglia (Reichmann et al., 2002). Some data suggest that dendritic cells may carry antigen from the CNS into the regional lymph
nodes and may trigger specific immune responses (de Vos et al., 2002; Karman et al., 2004). In addition, they are potent sources of proinflammatory cytokines and may preferentially attract CD8+ T-cells into the brain tissue (Carson et al., 1999). It has to be emphasized, however, that, with the exception of LCH, dendritic cells within the brain compartment do not express CD1a and so are not derived from the pool of Langerhans cells (Plumb et al., 2003). It is therefore not clear whether Langerhans histiocytes, when they reach the brain compartment, behave in a way similar to that described above.

The neurodegeneration observed in the vicinity of granulomas located in extraaxial spaces, in the meninges and infundibulum, was a striking finding. In particular, the pathogenesis of diabetes insipidus and anterior pituitary hormone deficiencies may be explained by these neurodegenerative changes in the infundibulum, which may interfere with the axonal transfer of vasopressin or hormones from the hypothalamic nuclei to the pituitary, in addition to possible direct damage by the granuloma itself.

The nature and pathogenesis of the ‘neurodegenerative’ brain lesions in LCH patients have so far been unresolved and controversial. Although the presence of some inflammatory infiltrates has been described mainly in the brain tissue surrounding a granuloma (Kepes, 1979), and variable numbers of T-lymphocytes were found in the previous study by Grois et al. (1994), the profound inflammation that we observed in the present series of patients has not been recognized before (Feigin, 1956; Kristensson, 1966; Yamaguchi et al., 1972; Poe et al., 1994). In contrast to our findings, diffuse neurodegenerative lesions in LCH have been described as multiple sclerosis-like demyelinated plaques (Davison, 1933; Feigin, 1956; Kristensson, 1966; Yamaguchi et al., 1972; Kepes, 1979; Poe et al., 1994).

In all patients of our study, with a long observation period, neurodegenerative pathology was associated with significant neurological impairment. In all these cases a diffuse inflammatory infiltration of the tissue, mainly composed of CD8+ T-lymphocytes, and profound microglia activation was found. Many of the T-cells contained Granzyme B-reactive granules, suggesting cytotoxic activation. In contrast to previous studies, none of the lesions studied in our material showed multiple sclerosis-like primary demyelination, but revealed massive neuronal and axonal destruction with secondary demyelination. The pathological process in LCH neurodegenerative lesions is thus fundamentally different from that in multiple sclerosis and more closely resembles that found in paraneoplastic encephalitis or Rasmussen’s encephalitis (Bien et al., 2000; Bernal et al., 2003).

There are several potential mechanisms to account for the occurrence of inflammatory brain damage in LCH. Dendritic cells are potent sources of proinflammatory cytokines (Kannourakis and Abbas, 1994; Egeier et al., 1999). The local production of such cytokines in the periphery of granulomas may create an environment that allows the recruitment of inflammatory cells from the circulation into the brain. This concept may explain the inflammatory component within and around LCH granulomas, but is difficult to reconcile with the ongoing inflammation in chronic lesions lacking Langerhans histiocytes. Possibly, Langerhans histiocytes in the brain tissue partially de-differentiate and lose their specific markers, but still remain capable of producing proinflammatory cytokines.

Alternatively, LCH granulomas in the brain could harvest liberated neuronal antigens and stimulate an autoimmune response to brain tissue components or against antigens shared by histiocytes and microglia. Then the granuloma would trigger an autoimmune response, which may persist, even when all Langerhans histiocytes have been cleared from the brain lesions. The absence of Langerhans histiocytes in neurodegenerative brain lesions noted in earlier studies (Kepes, 1979; Poe et al., 1994) and the similarity of these lesions with those found in paraneoplastic conditions support the autoimmune hypothesis.

Pronounced inflammation was noted in all types of CNS disease in LCH, in early and late forms, as well as in granulomatous or diffuse lesions. This striking finding of our study might give direction to future treatment approaches for the CNS manifestations of LCH.

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