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Human cytomegalovirus infection in foci of Langerhans cell histiocytosis

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Abstract Langerhans cell histiocytosis (LCH) has been thought to be a disorder of immune regulation, and increasingly, evidence showing that the tissue damage in LCH involves lymphokines and pro-inflammatory cytokines is reported. We detected human cytomegalovirus (HCMV)-DNA in LCH cells in the foci of LCH lesions by immunohistochemistry, in situ hybridization and PCR. HCMV was detected in the nuclei and/or cytoplasm of LCH cells in 9 of 27 LCH cases by immunostaining. HCMV was probably an early antigen. In situ hybridization revealed signals for HCMV-DNA only in the nuclei of LCH cells in 10 of the 27 LCH cases. PCR analysis was performed in 20 of the LCH cases, and HCMV-DNA was detected in 7 of these. All 7 positive cases were also positive for HCMV by ISH and IHC. These findings suggested that early phase infection or reactivation of HCMV occurred in the LCH lesions. HCMV infection may be accompanied by impaired cytokine production. Our study also suggested a relationship between HCMV infection and expression of TNFα. In tissues affected by LCH, dermatopathic lymphadenopathy or malignant fibrous histiocytoma and in normal tissues no signals for Epstein-Barr virus-RNA were detected. These findings suggest that in some cases LCH is associated with HCMV infection.

Key words Langerhans cell histiocytosis · Cytomegalovirus · In situ hybridization · PCR · Cytokine

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Introduction

Langerhans cell histiocytosis (LCH) is a proliferative disorder of Langerhans cells. Dendritic cells including Langerhans cells and/or interdigitating cells are found in the skin, lymphoid tissues and other organs in LCH [5, 26]. Langerhans cells are potent antigen-presenting cells that play an important part in detecting foreign antigens that enter the body. LCH lesions involve bone, bone marrow, lungs, liver, lymph nodes, spleen, skin and/or the central nervous system. Various types of inflammatory cells are usually present with LCH cells in the foci of LCH lesions.

LCH has frequently been considered to be a disorder of immune regulation rather than a form of neoplasia [6, 24, 25, 27]. Recent studies, however, have demonstrated that LCH may be a clonal neoplastic disorder with highly variable biological behaviour and clinical severity [38, 39]. The histological features of LCH also suggest that cytokines are involved in its pathogenesis [2, 14]. There is increasing evidence of the involvement of lymphokines and pro-inflammatory cytokines in the tissue damage seen in LCH. A number of viruses may cause affected cells to release cytokines, which could ultimately stimulate Langerhans cell growth. Exposure to viruses has been postulated to activate the immune system [8, 16].

We hypothesized that this disorder might be associated with viruses such as human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), herpes simplex virus, human herpesvirus 6 and retroviruses. In this study, we focused on herpesviruses.

Materials and methods

Twenty-seven cases of Langerhans cell histiocytosis (LCH) were selected from the archives of the Pathology Division, Kitasato University Hospital and Saitama paediatric Medical Centre. The patients consisted of 10 males and 17 females, being 21 children and 6 adults. One of the cases was an autopsy. Other clinical features are listed in Table 1. Control tissues included 18 cases of dermatopathic lymphadenopathy (DL) and 13 malignant fibrous

Table 1 Clinical features of the patients with Langerhans cell histiocytosis (*M* male, *F* female, *Lu* lung, *li* liver, *GI* gastrointestinal, *sp* spleen, *LN* lymph node)

Case	Sex	Age (years)	Sites of disease	Tissue examined	Therapy[b]
1	F	2	Lu, li, GI tract, sp, LN, skin	Lung, LN	Yes
2	M	2	Femur	Bone	No
3	M	11	Clavicle	Bone	No
4	M	1	Skull	Bone	No
5	M	5	Skull	Bone	No
6	M	9/12	LN	LN	No
7	F	2	Skull	Bone	No
8	M	38	Skin	Skin	No
9	F	63	Parotid	Soft tissue	No
10	F	3	Skull	Bone	No
11	F	59	Skin	Skin	No
12	F	5	Skull	Bone	No
13	F	7	Skull, vertebra, pubis, femur	Bone	No
14	F	9/12	Zygoma, soft tissue[a]	Bone	No
15	F	63	Tongue	Tongue	No
16	F	9	Skull	Bone	No
17	M	5/12	Skin	Skin	No
18	M	9	Skull, skin	Soft tissue	No
19	F	8	Skin	Skin	No
20	F	39	LN	LN	No
21	M	13	Skull, skin[a]	Skin	No
22	F	2	Scapula	Bone	No
23	F	1	Femur	Bone	No
24	M	3	Femur	Bone	Yes
25	F	26	LN	LN	No
26	F	6	Femur	Bone	No
27	F	9	Skull	Bone	No

[a] Invasive to soft tissue and skin
[b] Chemo- or radiotherapy before surgical treatment

histiocytomas (MFH). Moreover, normal tissues (7 samples each of salivary glands, liver, lung, kidney, tonsil, lymph node and skin, and 15 pancreatic samples) were also examined for detection of HCMV genome by ISH and PCR methods. The specimens obtained at biopsy, surgical resection and/or autopsy were fixed in 10% formalin and embedded in paraffin.

Sections 4 µm thick from formalin-fixed, paraffin-embedded tissues were mounted on silane-coated slides, air-dried and heated at 60° C for 30 min before immunohistochemical staining. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in methanol for 30 min. After treatment with 2% normal swine serum in phosphate-buffered saline, the tissue sections were incubated overnight with monoclonal anti-human cytomegalovirus (HCMV) antibodies (CCH2, Dako, Glostrup, Denmark and NCL-CMV-EA and -LA, Novocastra, Newcastle, UK), anti-S100 protein antibody (Dako, Calif.), monoclonal anti-CD1a (Immunotech, Marseille, France), anti-CD4 antibodies (Novocastra, Newcastle, UK), polyclonal anti-tumour necrosis factor alpha (TNFα, R&D system, Minn.) and/or polyclonal anti-lysozyme antibody (Dako, Calif.) at room temperature. The CCH2 monoclonal antibody recognizes both early and late antigens of HCMV [29, 40]. NCL-CMV-EA and -LA monoclonal antibodies react with early and late antigens of HCMV, respectively. The sections were incubated with biotinylated anti-mouse or -goat IgG (Vector Lab., Calif., 1:200 dilution) at room temperature for 30 min and then with streptavidin–biotin–peroxidase complex at 1:100 dilution (Amersham International, Bucks., UK). The peroxidase reaction was carried out with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (Dojindo, Kumamoto, Japan) containing 0.006% hydrogen peroxide in 0.05 mM Tris buffer, pH 7.6. As a positive control for HCMV, lung tissue infected with HCMV from a patient suffering from malignant lymphoma was used. Negative control staining was conducted in the absence of the primary antibody to HCMV. The sections were counterstained with haematoxylin and/or methyl green. Immunohistochemical staining for HCMV was assessed as positive when nuclei were stained with or without cytoplasmic staining.

For in situ hybridization, sections were deparaffinized with xylene and passed through a graded alcohol series and rehydrated with standard sodium citrate (SSC) solution, then digested with 0.2% pepsin (Sigma, Mo.) in 0.2 N HCl at 37° C for 30 min. After denaturation at 92° C for 30 min the alkaline phosphatase-labelled oligonucleotide probe for HCMV (Dako, Calif.) was applied to the sections at 50° C for 30 min and washed with SSC solution. As a positive control, the same HCMV-infected lung tissue was used for immunostaining. Epstein-Barr virus (EBV) RNA in situ hybridization was also performed according to Kasai et al. [15] using a biotinylated Epstein-Barr-encoded small RNA 1 (EBER-1) (Research Genetics, Ala.) probe. The tissue sections were deparaffinized, rehydrated, pretreated with 1 µg/ml proteinase K (Sigma, Mo.) at 37° C for 10 min, and hybridized with the probe for 30 min min at 42° C. After washing, the slides were incubated with streptavidin–alkaline phosphatase solution (Research Genetics, AL, USA) for 60 min at 37° C, washed, and incubated with a solution containing 5-bromo-4-chloro-3-indoyl phosphate (BCIP) and nitroblue tetrazolium (NBT; both from Sigma, Mo.) for 5 min at 42° C. After washing with distilled water, the slides were counterstained with methyl green. As a positive control, tissue sections of an EBV-positive anaplastic large cell lymphoma was employed. HCMV positivity was assessed in the same way as for immunohistochemical analysis.

DNA was extracted from formalin-fixed, paraffin-embedded tissue as previously described [21]. Briefly, five 10-µm paraffin sections of each specimen were placed in 1.5-ml Eppendorf tubes, and DNA was extracted as follows. The sections were deparaffinized with 1 ml of xylene for 5 min at room temperature and centrifuged at 12,000 *g* for 10 min, and the supernatant was then decanted. The pellet was washed with absolute ethanol followed by centrifugation at 12,000 *g* for 10 min and the supernatant decanted. This procedure was repeated three times. The pellet was air-dried and resuspended in 300 µl of 15 mM trisodium citrate solution containing 150 mM sodium chloride, 1% sodium dodecyl sulfate, and 300 µg/ml of proteinase K and incubated at 48° C for 48 h. DNA extracted from the formalin-fixed, paraffin-embedded lung tissue from the patient with HCMV pneumonitis described above was used as a positive control.

For amplification of the HCMV genome, primers were synthesized to amplify a 152-bp segment in the AD169 EcoRI fragment D region of the HCMV genome (sense primer 5′-GATCCGACCATTGTCTAAG-3′ and antisense primer 5′-GGCAGCTATCGTGGGA-3′) [10]. Amplification was carried out by the method of Saiki et al. [31]. Briefly, 1-µg DNA samples were diluted in 100 µl of a solution containing 200 µM deoxynucleotide triphosphates (dNTP), 1 µM of each primer, 2.5 U of Ampli Taq DNA polymer-

Table 2 Results of immunohistochemistry (*IHC*) in situ hybridization (*ISH*) and polymerase chain reaction (*PCR*) in foci of Langerhans cell histiocytosis. (*Lyso* lysozyme, *P* positive, *N* negative, *ND* not done, *FA* final assessment for HCMV positivity)

Case	Sex	Age	IHC						ISH		PCR	FA
		(years)	S100	CD1a	CD4	Lyso	TNFα	HCMV	HCMV	EBER1	HCMV-DNA	
1	F	2	p	p	N	N	P	P	P	N	P	P
2	M	2	p	p	N	N	P	N	N	N	N	N
3	M	11	p	p	N	N	N	N	N	N	N	N
4	M	1	p	p	N	N	P	N	N	N	N	N
5	M	5	p	p	N	N	P	N	N	N	ND	N
6	M	9/12	p	p	N	N	N	N	N	N	N	N
7	F	2	p	p	N	N	N	N	N	N	ND	N
8	M	38	p	p	N	N	N	P	P	N	ND	N
9	F	63	p	p	N	N	ND	N	N	ND	ND	N
10	F	3	p	p	N	N	P	N	P	N	N	N
11	F	59	p	p	N	N	P	P	P	N	P	P
12	F	5	p	p	N	N	N	N	N	N	ND	N
13	F	7	p	p	N	N	P	N	N	N	N	N
14	F	9/12	p	p	N	N	ND	N	N	N	N	N
15	F	63	p	p	N	N	N	N	N	N	ND	N
16	F	9	p	p	N	N	P	P	P	N	P	P
17	M	5/12	p	p	N	N	N	N	N	N	N	N
18	M	9	p	p	N	N	P	N	N	N	N	N
19	F	8	p	p	N	N	ND	N	N	N	ND	N
20	F	39	p	p	N	N	N	P	P	N	P	P
21	M	13	p	p	N	N	P	P	N	N	N	N
22	F	2	p	p	N	N	N	N	N	N	N	N
23	F	1	p	p	N	N	P	P	P	N	P	P
24	M	3	p	p	N	N	N	N	N	N	N	N
25	F	26	p	p	N	N	P	P	P	N	N	N
26	F	6	p	p	N	N	P	P	P	N	P	P
27	F	9	p	p	N	N	P	P	P	N	P	P

Table 3 Results of immunohistochemistry, in situ hybridization and PCR in dermatopathic lymphadenopathy (*DL*), malignant fibrous histiocytoma (*MFH*) and normal tissues (*Nor* normal tissue, *TNF* tumour necrosis factor alpha, *HCMV* human cytomegalovirus, *EBER1* Epstein-Barr-encoded small RNA 1, * remaining tissues were not examined)

	IHC						ISH		PCR
	S100	CD1a	CD4	Lyso	TNF	HCMV	HCMV	EBER1	HCMV
DL	16/16	16/16	0/16	0/16	3/16	0/16	0/16	0/16	0/8*
MFH	3/13	ND	ND	ND	7/13	0/13	0/13	0/13	0/7*
Nor	ND	ND	ND	ND	ND	ND	0/63	ND	0/60*

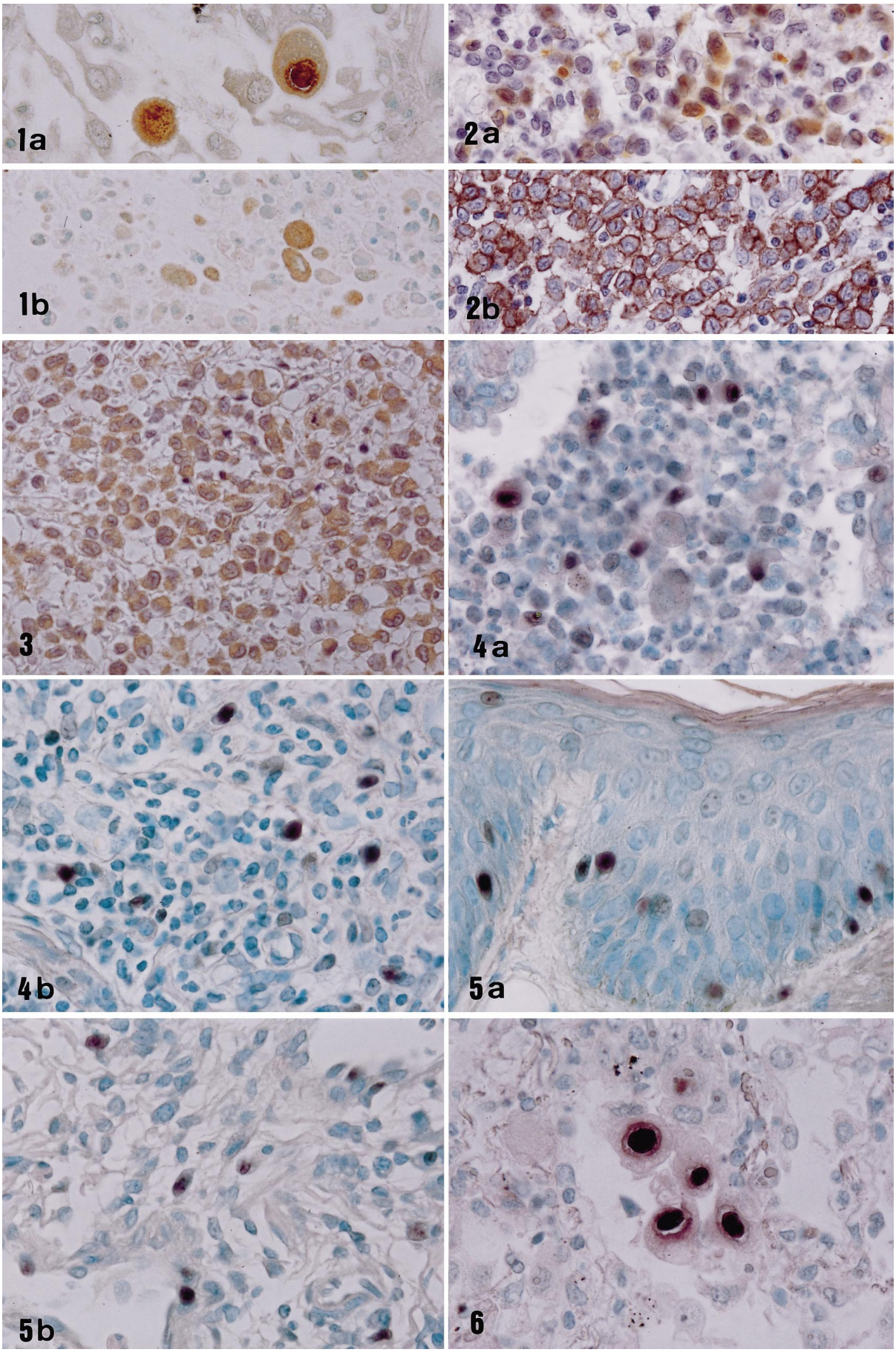
ase, and 1×PCR buffer (both from Takara, Shiga, Japan). Negative controls were prepared using the same reaction mixture and distilled water instead of extracted DNA. PCR was performed as follows; 5 min at 94° C for DNA denaturation, followed by 30 cycles consisting of denaturation for 30 s at 94° C, annealing for 30 s at 55° C, and extension for 30 s at 72° C. For the last PCR cycle, the DNA was allowed to extend for 7 min at 72° C. The amplified products were electrophoresed in 3% agarose gels containing ethidium bromide in Tris-borate-EDTA buffer. After electrophoresis, the gels were photographed under ultraviolet light.

Cases were assessed as positive when HCMV was detected in all three of the analyses, that is to say by immunohistochemistry, in situ hybridization and PCR methods, to prevent the possibility of false-positive or false-negative results.

Results

Most (25) of the 27 LCH patients did not receive chemo- or radiotherapie before surgical treatment. Patient 1, who received chemotherapy with steroid hormones for 6 weeks, received no further treatment until death. Patient 24 had neuroblastoma, and was treated with cyclophosphamide, pirarubicin and carboplatin, and 3 years later developed LCH in the left femur. In 25 of the 27 cases, the lesions were localized. The exceptions were cases 1 and 13. In patient 1, LCH lesions were seen in several organs, including the lungs, liver, gastrointestinal tract, spleen, lymph nodes and skin. In patient 13, the lesions were also seen in multiple sites (Table 1). LCH cells in all foci were generally accompanied by eosinophils, lymphocytes, macrophages and giant cells.

In the disseminated HCMV-infected case examined as a positive control, immunoreactive antigen and ISH-directed DNA for HCMV were colocalized in the same cells (Fig. 1a). HCMV antigen was detected in both nuclei and/or cytoplasm of LCH cells in 9 of the 27 cases


1a
2a
1b
2b
3
4a
4b
5a
5b
6

(Table 2, Fig. 1b). HCMV early antigen was detected in both LCH and the positive control. In most cases positive for HCMV, fewer LCH cells were positive by immunostaining than by in situ hybridization. All of the specimens were positive for S100 protein and CD1a on paraffin sections of LCH cells in LCH cases (Fig. 2a, b) and dendritic or interdigitating cells in DL cases (Tables 2, 3), while 3 of 13 cases of MFH expressed S100 protein. TNFα was detected in 14 of 24 LCH cases, 3 of 16 DL cases and 7 of 13 MFH cases (Tables 2, 3). The cytokine was detected in 58% of the patient irrespective of positivity or negativity for HCMV. However, strong expression of TNFα was observed in the LCH cells in 5 of the 7 cases that were positive for HCMV (Fig. 3), and the number of negative cases remaining at the final assessment was out of 16. Neither CD4 nor lysozyme was detected in any case of LCH and DL (Tables 2, 3).

In in situ hybridization, signals for HCMV-DNA were detected only in the nuclei of LCH cells in 10 of the 27 LCH cases. In most cases, the signals were detected in a few LCH cells (Fig. 4a, b). Moreover, in patient 11, HCMV was detected not only by ISH but also by IHC in both epidermal Langerhans cells (LCs) and LCH cells (Fig. 5a, b). In the HCMV-positive control, signals for HCMV-DNA were seen in both nuclei and cytoplasm of the cells with cytomegalic inclusions (Fig. 6). No signals for HCMV were detected in DL, MFH or normal tissue. No signals for EBV-RNA were detected by ISH in LCH, DL, MFH or normal tissues.

PCR was performed in 20 of the 27 LCH cases, in 7 of which HCMV-DNA was detected (Fig. 7). All 7 positive cases were also positive for HCMV by ISH and IHC. HCMV-DNA in cytomegalic cells was detected in several organs in the HCMV-disseminated control. DL, MFH and normal tissues were negative for HCMV-DNA on PCR analysis in all the tissues examined. The final frequency of HCMV positivity in LCH foci was 7 of the 27 (25.9%) cases.

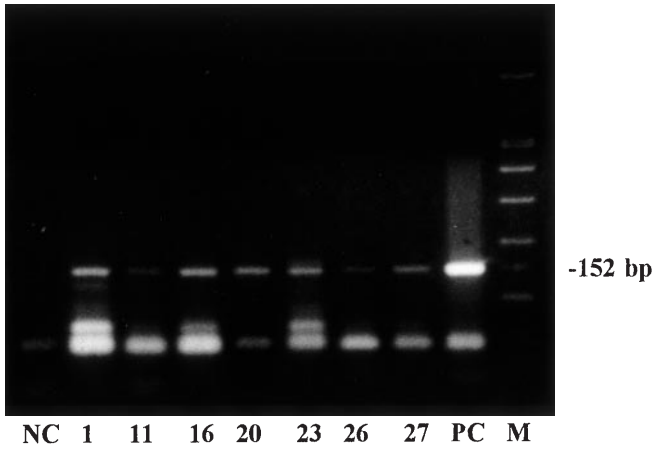


Fig. 7 Agarose gel electrophoresis of amplified PCR products for HCMV-DNA fragments extracted from LCH and HCMV-positive control tissues. *Lane NC* negative control, *lanes 1–8*, extracts from foci of LCH cases 1, 11, 16, 20, 23, 26 and 27, *lane PC* positive control (extracts from the lung of the HCMV-disseminated control) showing a band of 152 bp, *lane M*; PCR molecular weight markers (Amersham Life Science, Ohio). The *lower bands* represent oligonucleotide primer dimers

Discussion

Seven of the 27 cases of Langerhans cell histiocytosis (LCH) were shown to be positive for human cytomegalovirus (HCMV) by in situ hybridization (ISH), immunohistochemistry (IHC) and PCR. With the exception of patient 1, the HCMV-positive patients did not receive prior immunosuppressive therapy. Therefore, the possibility that HCMV infection or reactivation was due to therapy could be excluded at least in the remaining 6 cases. Dermatopathic lymphadenopathy (DL), malignant fibrous histiocytoma (MFH) and normal tissues were negative for HCMV infection by any analytical method used. Moreover, Epstein-Barr virus was not detected in any of the cases in the present study, including LCH lesions and/or normal tissues. In the 9 LCH lesions, HCMV was found by IHC to be an early antigen. This suggests the early phase of infection or reactivation of HCMV, as the immediate early and/or early antigen is well known to appear during active infection or reactivation of HCMV [33, 34, 37]. In the present study, however, it was unclear whether viral infection or reactivation occurred before or after the proliferation of LCH cells. HCMV can cause infections in immunocompromised patients. Owing to the high incidence of latent HCMV infection in healthy subjects, clinically apparent HMCV infection frequently results from reactivation of the latent infection, but the mechanism of reactivation is unknown. On the other hand, we also detected HCMV by IHC and ISH in epidermal LCs in neighbouring LCH foci. However, it was not clear from these findings is whether the HCMV in the epidermal LCs was transmitted from LCH cells in the foci or was already present in the LCs before the development of LCH lesions. There have been vari-

Fig. 1. HCMV (human cytomegalov antigen is seen in both nuclei and cytoplasm **a** of disseminated HCMV-infected positive control tissue and **b** of LCH cells from case 27 on immunohistochemical staining

Fig. 2 Immunohistochemical expression of **a** S100 protein and **b** CD1a in LCH cells (case 16)

Fig. 3 Immunohistochemical expression of TNFα in Langerhans cell histiocytosis (LCH) cells (case 11)

Fig. 4 On in situ hybridization, signals for HCMV-DNA were observed only in the nuclei of LCH cells of the lung **a** in case 16 (as in **Fig. 2a** and **b** in case 20

Fig. 5 Signals for HCMV-DNA on ISH **a** in epidermal Langerhans cells (LCs) and **b** in the neighbouring LCH foci of case 11

Fig. 6 Signals for HCMV-DNA were seen in both nuclei and cytoplasm of cells with cytomegalic inclusions in HCMV-positive control

ous reports [1, 4, 13, 19, 28, 35] showing that epidermal cells, fibroblasts, monocytes and macrophages can be infected with viruses, including HCMV, depending on the stage of cell differentiation. Peripheral leucocytes, especially monocytes, appear to harbour the latent virus in healthy seropositive individuals [35]. After infection of monocytes, macrophages resident in tissues may contain low levels of HCMV. However, there is no evidence that LCs are infected.

HCMV infection is accompanied by impaired cytokine production [22]. For instance, patients with HCMV diseases have high plasma levels of TNFα [32, 36] and IL-6 [11, 12]. Docke et al. [3] reported that HCMV antigenaemia and enhanced plasma. TNFα occurred in many patients with liver cirrhosis and immunodeficiency. They suggest that TNFα plays a central role in HCMV reactivation. Moreover, TNF seems to be the most important cytokine involved in activation of the HCMV immediate-early (IE) promoter [7], and transcriptional regulation of the HCMV IE promoter is apparently mediated by factors produced by macrophages or macrophage lineage cells [17, 30].

Nevertheless, the findings that TNFα and GM-CSF stimulate the growth of LCs in vitro have generated a great deal of interest in these cytokines in LCH lesions. The higher cytokine production by LCH cells than normal epidermal LCs may reflect the activated state of these cells involved in this disease [41]. Kannourakis et al. [14] reported that IL-1, TNFα and GM-CSF levels were slightly or significantly elevated according to both immuno- and bioassays of sera from patients with active LCH. We speculated that such cytokine production by LCH cells and/or lymphocytes might be accelerated directly or indirectly by infection with HCMV. In the present study, TNFα was detected in 14 of 24 LCH cases irrespective of positive or negative HCMV status. Our study suggested a relationship between HCMV infection and TNFα, because strong expression of TNFα was observed in 5 of the 7 cases that were HCMV positive and the number of negative cases remaining at the final assessment was 9 out of 17. In contrast, in DL and MFH the expression was weak, although a large series was not examined.

Viral infections such as HCMV, human herpesvirus 6 and vaccinia virus have been reported to influence LC distribution in tissues [9, 18, 23]. Hesseling et al. [9] also speculated that both monocyte/macrophage disorder and dendritic disease are triggered by the virus or by virus-associated cytokines secreted by activated macrophages. In addition, McClain et al. [20, 21] examined the possible aetiological link between viruses and LCH. They searched for evidence of viral infection of lymphocytes or histiocytes in the lesions of LCH patients, using probes for nine different viruses that commonly infect children and affect the immune system. However, they found no correlation between viral infection and development of LCH lesions. The reason for the discrepancy between these results and those of the present study is unclear. However, viruses including HCMV may not persist in LCH cells or an any site, and the discrepancy may have been due to differences in methodology. (The ISH procedures, primers and probe used by McClain et al. were different from those used in the present study.)

We detected HCMV genome in LCH cells at a high frequency by ISH, IHCand PCR. On the basis of our results, we speculate that HCMV may be associated with the pathogenesis of LCH. However, other possible causes, such as autocrine and/or paracrine secretion of growth factors, lesions of proliferation of cells in LCH, and the possibility of other viral infections cannot be excluded. Further studies are required before we can understand the mechanisms involved in the development and homing of dendritic cells and the expression of cytokines in LCH lesions.

References

1. Berger R, Gartner S, Rapersberger K, Foster CA, Wolff K, Stingl G (1992) Isolation of human immunodeficiency virus type I from human epidermis: virus replication and transmission studies. J Invest Dermatol 99:271–277
2. De Graaf JH, Mannes GP, Tamminga RY, Kamps WA, Timens W (1995) The role of cytokines in the pathogenesis of pulmonary Langerhans' cell histiocytosis. Adv Exp Med Biol 378: 535–537
3. Docke WD, Prosch S, Fietze E, Kimel V, Zuckermann H, Klug C, Syrbe U, Kruger DH, von Baehr R, Volk HD (1994) Cytomegalovirus reactivation and tumor nectosis factor. Lancet 343:268–269
4. Dudding L, Haskill S, Clark BD, Auron PE, Sporn S, Huang ES (1989) Cytomegalovirus infection stimulates expression of monocyte-associated mediator genes. J Immunol 143:3343–3352
5. Favara BE (1991) Langerhans cell histiocytosis. Pathobiology and pathogenesis. Semin Oncol 18:3–7
6. Favara BE, McCarthy RC, Mierau GW (1983) Histiocytosis X. Hum Pathol 14:663–676
7. Fietze E, Prosch S, Reinke P, Stein J, Docke WD, Staffa G, Loning S, Devaux S, Emmrrich F, von Baehr R, Kruger DH, Volk HD (1994) Cytomegalovirus infection in transplant recipients. The role of tumor necrosis factor. Transplantation 58: 675–680
8. Greaves MF, Alexander FE (1993) An infectious etiology for common acute lymphoblastic leukemia in childhood? Leukemia 7:349–360
9. Hesseling PB, Wessels G, Egeler RM, Rossouw DJ (1995) Simultaneous occurrence of viral-associated hemophagocytic syndrome and Langerhans cell histiocytosis: a case report. Pediatr Hematol Oncol 12:135–141
10. Hsia K, Spector DH, Lawrie J, Spector SA (1989) Enzymatic amplification of human cytomegalovirus sequences by polymerase chain reaction. J Clin Microbiol 8:1802–1809
11. Humbert M, Devergne O, Cerrina J, Rain B, Simonneau G, Dartevelle P, Duroux P, Galanaud P, Emilie D (1992) Activation of macrophages and cytotoxic cells during cytomegalovirus pneumonia complicating lung transplantations. Am Rev Respir Dis 145:1178–1184
12. Humbert M, Delattre RM, Fattal S, Rain B, Cerrina J, Dartevelle P, Simonneau G, Duroux P, Galanaud P, Emilie D (1993) In situ production of interleukin-6 within human lung allografts displaying rejection and cytomegalovirus pneumonitis. Transplantation 56:623–627
13. Ibanez CE, Schrier R, Ghazal P, Wiley C, Nelson JA (1991) Human cytomegalovirus productively infects primary differentiated macrophages. J Virol 65:6581–6588
14. Kannourakis G, Abbas A (1994) The role of cytokines in the pathogenesis of Langerhans cell histiocytosis. Br J Cancer 70: S37–S40

15. Kasai K, Sato Y, Kameya T, Inoue H, Yoshimura H, Kon S, Kikuchi K (1994) Incidence of latent infection of Epstein-Barr virus in lung cancers−pananalysis of EBER-1 expression in lung cancers by in situ hybridization. J Pathol (Lond) 174:257–265
16. Kinlen LJ (1989) Infective cause of childhood leukemia. Lancet I:378–379
17. Lafemina R, Hyward GS (1986) Constitutive and retinoic acid-inducible expression of cytomegalovirus immediate-early genes in human teratocarcinoma cells. J Virol 58:434–440
18. Leahy MA, Krejci SM, Friednash M, Stockert SS, Wilson H, Huff JC, Weston WL, Brice SL (1993) Human herpesvirus 6 is present in lesions of Langerhans cell histiocytosis. J Invest Dermatol 101:642–645
19. Maciejewski JP, Bruening EE, Danahue RE, Sellers SE, Carter C, Young NS, St Jeor S (1993) Infection of mononucleated phagocytes with human cytomegalovirus. Virology 195:327–336
20. McClain K, Weiss RA (1994) Viruses and Langerhans cell histiocytosis: Is there a link? Br J Cancer 70 (S23):34–36
21. McClain K, Jin H, Gresik V, Favara B (1994) Langerhans cell histiocytosis: lack of a viral etiology. Am J Hematol 47:16–20
22. Michelson S, Alcami J, Kim SJ, Danielpour D, Bachelerie F, Picard L, Bessia C, Paya C, Virelizier JL (1994) Human cytomegalovirus infection induces transcription and secretion of transforming growth factor β1. J Virol 68:5730–5737
23. Nagao S, Inaba S, Iijima S (1976) Langerhans cells at the sites of vaccinia virus inoculation. Arch Dermatol Res 256:23–31
24. Newton W, Hamoudi A (1973) Histiocytosis: a histologic classification with clinical correlation. Perspect Pediatr Pathol 1: 252–257
25. Nezelof C (1979) Histiocytosis X: a histological and histogenetic study. Perspect Pediatr Pathol 5:153–178
26. Nezelof C, Basset F, Rousseau MF (1973) Histiocytosis X. Histogenetic argument for a Langerhans cell origin. Biomedicine 18:365–371
27. Osband ME, Lipton JM, Lavin P, Levey R, Vawter G, Greenberger JS,McCaffrey RP, Parkman R (1981) Histiocytosis X. Demonstration of abnormal immunity, T-cell histamine H2-recptor deficiency, and successful treatment with thymic extract. N Engl J Med 304:146–153
28. Ostertag W, Vehmeyer K, Fagg B, Pragnell IB, Paeta W, Le Bousse MC, Smadja-Joffe F, Klein B, Jasmin C, Eisen H (1980) Myeloproliferative virus, a cloned murine sarcoma virus with spleen focus-forming properties in adult mice. J Virol 33:573–582
29. Plachter B, Nordin M, Wirgart BZ, Mach M, Stein M, Grillner L, Jahn G (1992) The DNA-binding protein p52 of human cytomegalovirus reacts with monoclonal antibody CCH2 and associates with the nuclear membrane at late times after infection. Virus Res 24:265–276
30. Ramesh N, Shin YK, Lau S, Osborne WRA (1995) High-level expression from cytomegalovirus promoter in macrophage cells. Hum Gene Ther 6:1323–1327
31. Saiki RK, Gelfant DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491
32. Smith PD, Saini SS, Raffeld M, Manischewitz JF, Wahl SF (1992) Cytomegalovirus induction of tumor necrosis factor-alpha by human monocytes and mucosal macrophages. J Clin Invest 90:1642–1648
33. Stenberg RM, Thomsen DR, Stinski MF (1984) Structural analysis of the major immediate early gene of human cytomegalovirus. J Virol 49:190–199
34. Swenson PD, Kaplan MH (1985) Rapid detection of cytomegalovirus in cell culture by indirect immunoperoxidase staining with monoclonal antibody to an nuclear antigen. J Clin Microbiol 21:669–673
35. Tailor-Wiedeman J, Sissons JGP, Borysiewicz LK, Sinclair JH (1991) Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. J Gen Virol 72:2059–2064
36. Tilg HW, Vogel WB, Aulitzky M, Herold A, Konigsrainer R, Margreiter R, Huber C (1990) Evaluation of cytokines and cytokine-induced secondary messages in sera of patients after liver transplantation. Transplantation 49:1074–1080
37. Van der Bij W, Trensma R, van Son WJ, Anema J, Schirm J, Tegzess AM, The TH (1988) Rapid immodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. J Med Virol 25:179–188
38. Willman CL (1994) Detection of clonal histioytes in Langerhans cell histiocytosis: Biology and clinical significance. Br J Cancer 70:S29–S33
39. Willman CL, Busque L, Griffith BB, Favara BE, McClain KL, Duncan MH, Gilliland DG (1994) Langerhans cell histiocytosis (histiocytosis X): a clonal proliferative disease. N Engl J Med 331:154–160
40. Wirgart BZ, Landqvist M, Hokeberg I, Eriksson B-M, Olding-Stenkvist E, Grillner L (1990) Early detection of cytomegalovirus in cell culture by a new monoclonal antibody, CCH2. J Virol Methods 27:211–220
41. Yu R, Buluwela L, Alaibac M, Chu A (1993) Cytokine expression by human epidermal Langerhans cells and Langerhans cell histiocytosis cells. Br J Dermatol 129(S42):29