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Western blot profiles, lymph node ultrastructure and viral expression in HIV-infected patients: a correlative study

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SUMMARY

Sequential immunoblotting was performed on 64 patients infected with human immunodeficiency virus (HIV). Antibody profiles were related to immune function, T subsets and clinical features. In 20 patients, lymph node biopsy revealed a relationship between progressive follicular destruction, low antibody titres and ultrastructural evidence of viral replication and accumulation. Retroviral particles, including budding profiles, were confined to labyrinths formed from hypertrophied follicular dendritic (FD) cells; in some cases, including those with AIDS, the labyrinths showed degenerative changes. The demonstration of high antibody levels in asymptomatic patients with an intact FD cell network and low virion load suggests that antibody may have a protective role *in vivo*. Analysis of lymph node ultrastructure allows assessment of viral load and FD cell morphology. When combined with immunoblotting, it may be possible to improve prognostic stratification of patients with HIV infection.

Keywords HIV serology lymph node ultrastructure AIDS follicular dendritic cell

INTRODUCTION

Immunoblotting against HIV viral lysate is the most commonly used confirmatory test for anti-HIV antibodies. This test remains poorly standardized and there is considerable variability in the profiles of seropositive patients. False positives and negatives have been reported and many tests may be regarded as indeterminate.

The reasons for the differences between subjects remain poorly understood. Differences in the duration or extent of infection may be important, but Safai *et al.* (1984) have suggested that degrees of antigen expression and viral proliferation may determine antibody profiles. Since we can correlate immunological data with ultrastructural evidence of the infection (Armstrong & Horne, 1984; Armstrong, Dawkins & Horne, 1985) and particularly the extent of viral sequestration in lymph nodes, we have examined some of these issues. Here we will show that there is a relationship between nodal ultrastructure, histopathology, and the HIV serology and that there is an inverse relationship between HIV antibody titres and evidence of viral replication.

MATERIALS AND METHODS

The 64 patients studied were referred to the Department of Clinical Immunology because of the

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suspicion of HIV infection. All were seropositive on at least one occasion. Most were homosexual but some had acquired infection via infusion of blood products. All were studied sequentially for up to 3 years. Patients were categorized clinically as having: (i) AIDS according to standard criteria (CDC, 1984); (ii) persistent generalized lymphadenopathy, defined as lymphadenopathy in more than two noncontiguous regions for more than 3 months; or (iii) asymptomatic seropositivity. Lymphocyte subsets were determined by direct immunofluorescence using monoclonal antibodies specific for CD4, CD8 and Ia/DR and analysed on cytofluorograph 50L (Ortho).

Serology. Western blotting was performed using the strip radioimmunoassay (RIA) method as outlined by Sarngadharan and co-workers (1984). In summary, HTLV-III viral lysate (NCI Batch S-2381), obtained by lysis of virus grown in H9 cell line in 1% Triton X-100 (TX-100) and 0·5% NaDOC with subsequent ether extraction three times, was run at 5 μg/lane on 12% polyacrylamide gel and transferred to nitrocellulose by the method of Towbin, Staehelin & Gordon (1979). Sheets were incubated in 5% BSA in Tris/saline and cut into 0·5 cm strips, incubated for 2 h at 37°C in 2·5 ml of buffer (0·3% TX-100 and 2 mg BSA/ml). Test sera (25 μl) were then added to individual tubes containing a strip and incubated for 1 h at room temperature (RT) and overnight at 4°C. Strips were washed three times with a solution containing 0·5% sodium deoxycholate, 0·1 M NaCl, 0·5% TX-100, 1 mm PMSF and 10 mm sodium phosphate pH 7·5 (buffer 1). Strips were then incubated for 1 h at RT with 2·4 ml buffer 1 and 0·1 ml of normal goat sera: affinity purified I^{125} labelled goat anti-human immunoglobulin ($1{\cdot}2 \times 10^6$ ct/min) was added and incubated for 30 min at RT, washed, dried and exposed to X-ray film (Kodak XAR5) overnight at −70°C. Control sera were run in serial dilution on each blot and used to standardize scoring systems and control for blot-to-blot variations in sensitivity. The enzyme linked immunoassay (ELISA) was done using commercial kits (Abbott or Litton).

Both Western blotting (Towbin & Gordon, 1984) and ELISA are quantitative assays. Using appropriate controls and standards it is possible to determine both composite and relative titres from optical density (OD) and band intensity. For Western blot bands we used the grading scale as suggested by Schupbach *et al.* (1985). This scale was then related to control dilution series run and analysed with each blot. Figure 2 illustrates the relationship of antibody titre to ELISA OD and Western blot band intensities.

Ultrastructural studies. Twenty patients underwent diagnostic lymph node biopsy. Enlarged axillary, inguinal or cervical nodes were fixed in either buffered 10% formalin for paraffin embedding and routine histopathology or 2·5% glutaraldehyde in 0·1 molar cacodylate buffer (pH 7·2) followed by osmication and embedding in epoxy resin as described previously (Armstrong & Horne, 1984). Staining of the tissue fragments *en bloc* with 2% aqueous uranyl acetate, before dehydration and embedding for thin sectioning, was useful to enhance the contrast of viral particles in the tissues. Specimens were viewed with a Philips EM410 (HM) electron microscope, multiple blocks being examined from each biopsy specimen; and were screened systematically to include follicular, interfollicular and medullary areas.

RESULTS

Analysis of bands detected by Western Blot. As can be seen in a representative blot of positive sera (Fig. 1), the intensity of different bands ranged considerably. Comparison with serial dilution of positive sera (Fig. 2) showed the score correlated with titre and there was early loss of 18 and 32 bands with subsequent loss of 40; residual bands consisted of 24, 44 and lesser 55. Those sera with the strongest bands have the most bands and a reduction in number of bands correlates with a reduction in band intensity. Where few weak bands were seen these included 24, 44, 55, 65; i.e. those bands detected in the highest dilution of positive sera. It appears that the reduced number of bands in weakly positive sera is a reflection of quantitative change rather than of qualitative difference in antibody specificity. This phenomenon is seen when patients are classified into three groups according to clinical status at the time sera were collected (Tables 1–3). Asymptomatic seropositive patients have the highest intensities and the highest banding scores, whereas patients with more advanced disease had lower antibody titres reflected in lower banding scores. Only a single patient

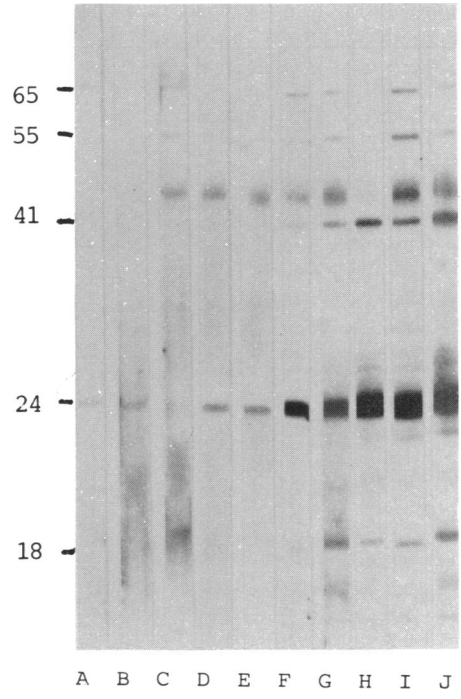

Fig. 1. Blot of seropositive subjects showing range of variation and relationship to clinical status. There are multiple strong bands in patients with high titre antibody (bands 65, 55, 44, 40, 24, 18). Reduction in intensity of bands and fewer bands are seen in sera with lower titres. Lanes F,G,H, asymptomatic; B,C, AIDS (cases Dhe and Kge, Table 4); A,D, persistent lymphadenopathy (cases Jhu and Gro, Table 4).

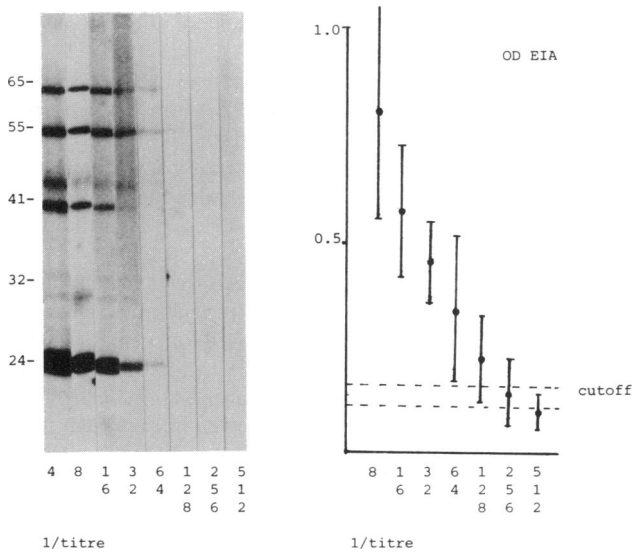

Fig. 2. Relationship of antibody titre to banding intensity and ELISA OD. Serum from patient Rwt (Table 4) was tested in doubling dilution in seronegative serum. Loss of the 40 and 44 bands occurred at a titre of 32 while 24, 55 and 65 extend to a titre of 256. These titres are reflected in population 'serographs' (Tables 1,2,3) where p24, 44, 55, and 65 are the most frequently identified bands.

Table 1. Asymptomatic seropositive

Patients	T4/8 ratio	EIA ratio	Western blot scores						
			44	24	55	65	18	40	32
Bra	0·8	>13	3	4	3	3	3	4	2
Njo	1·0	>13	3	4	2	2	3	3	2
Pwa	0·6	>13	3	4	2	2	3	3	2
Dwa	0·6	>13	4	4	2	2	2	4	1
Sra	1·4	>13	2	4	2	2	2	3	1
Dco†	1·2	>13	3	4	2	2	—	3	1
Hca	0·7	>13	2	4	2	2	—	3	—
Pbr†	0·8	>13	3	4	1	1	2	3	1
Aro	0·4	11·5	1	4	1	1	2	2	—
Jcl*	1·1	>13	3	4	1	1	1	2	—
Kme	1·2	3·2	1	4	1	1	1	2	—
Bhy	0·5	8·6	2	4	1	1	—	2	—
Rfe*	0·7	7·7	2	3	1	1	—	3	—
Jml	1·2	>13	1	3	2	2	1	2	—
Dhu	0·8	10·1	2	3	2	2	1	1	—
Dwi	0·2	10·3	2	2	1	2	1	—	1
Mca†	1·1	8	2	2	1	1	1	1	—
Mda	0·7	7·7	1	2	2	2	—	—	—
Khi	0·9	10·4	2	1	1	1	—	—	—
	0·8±0·3	11·0±2·8	2·2	3·1	1·6	1·7	1·1	2·9	0·5

* Hepatitis B carrier.
† Haemophiliac.

with AIDS (an African female with lymphadenopathic Kaposi's sarcoma) had all bands detected, while one-third of asymptomatic patients with lymphadenopathy had all viral specific bands detected.

Seroconversion and anti-p24 antibodies. Three of the patients studied seroconverted during the course of the study and sequential blots are shown (Fig. 3). All three had a strong 24 band initially but later developed a full range of bands. One of the three (Jco) seroconverted 2 months after massive blood transfusion for multiple trauma. He subsequently developed lymphadenopathy. One of the remaining two patients developed persistent lymphadenopathy. Four other patients had a single or predominant 24 band on initial testing and historical evidence of recent onset of febrile illness associated with lymphadenopathy.

Ultrastructural studies. Lymph node biopsy was performed in a total of 20 patients with generalized lymphadenopathy or AIDS (Table 4). The ultrastructural changes described with HIV-associated lymphadenopathy (Armstrong & Horne, 1984; Armstrong, Dawkins & Horne, 1985) were found in all these biopsies (Figs 4–8). These included germinal centre expansion with follicular dendritic (FD) cell hypertrophy and in some cases lytic degeneration. Retrovirus-like particles (90–120 nm diameter) with asymmetrical or discoid core profiles were present in variable numbers in these cases. Virions were associated with FD cells and located within the extracellular compartment of labyrinths formed by extensions and processes of these cells. Budding profiles, when present, were invariably found on cytoplasmic processes and surface membranes of the FD cells.

In contrast, prolonged search revealed no comparable images indicative of permissive retrovirus infection amongst the nodal lymphocyte populations, monocytes, histiocytes, interdigitating (interfollicular) dendritic cells or Langerhans cells. Productive replication with release of whole virions by such cell types, if occurring *in vivo*, would seem to be comparatively uncommon and so difficult to detect by electron microscopy.

Table 2. Lymphadenopathy cases

Patients	T4/8 ratio	EIA ratio	Western blot scores 44	24	55	65	18	40	32
Gts*	2·1	>13	3	4	3	3	3	4	1
Dbr	0·7	>13	4	4	2	2	3	4	1
Dbu†	0·3	>13	3	4	2	2	3	4	2
Rtr	0·8	>13	4	4	3	3	2	4	2
Lba	1·1	>13	4	4	3	3	2	4	2
Dar*	0·8	>13	1	4	2	2	3	4	1
Jwa	0·3	>13	4	4	2	2	2	4	2
Mwi	0·8	>13	2	4	2	2	2	3	2
Mbu	0·5	>13	3	4	2	2	2	3	1
Jco	0·5	>13	3	4	2	2	2	3	—
Spo	0·4	>13	3	4	2	2	1	2	—
Mml	0·5	3·9	3	4	3	2	—	1	—
Str	0·5	4·9	1	4	1	1	1	3	1
Dyo	0·6	12·0	2	4	1	1	1	3	—
Nsm	0·5	5·3	2	3	1	—	2	2	—
Dty	2·4	11·8	1	3	1	2	—	1	—
Jlo	0·8	>13	1	3	1	2	1	1	—
Vbe	0·5	>13	2	3	1	1	—	1	—
Ami	0·8	11·1	2	3	2	2	1	—	—
Jhu	0·7	>13	3	2	2	2	1	—	—
Kdi‡	0·5	11·6	1	1	3	3	1	—	—
Rda	0·4	10·2	2	1	3	3	1	—	—
Ish§	0·5	11·2	2	2	1	1	1	—	—
Rjo	0·9	>13	3	2	1	1	1	—	—
Iel‡‖	0·3	7·4	2	3	1	1	—	1	—
Cle†‖	0·3	12·7	1	3	1	1	—	—	—
Epa	0·3	10·1	2	2	1	2	—	—	—
Rpa	0·2	8·5	3	2	1	1	1	—	—
Rha	1·8	9·1	1	2	1	1	—	—	—
Gte	0·6	5·4	—	1	1	1	—	—	—
Ifr	0·3	10·4	—	1	1	—	1	—	—
Ble	0·5	10·3	1	2	—	—	1	—	—
Ado‡	0·7	7·4	2	1	3	3	—	—	—
Pco‡	0·5	2·8	1	2	—	—	—	—	—
Pno	2·2	11·8	1	2	—	—	—	—	—
Gro	0·7	7·8	1	1	—	—	—	—	—
	0·7±0·04	11·0±2·8	2·1	2·8	1·6	1·6	1·1	1·5	0·5

* Hepatitis B carrier.
† ITP.
‡ Constitutional symptoms (ARC).
§ Subsequently developed *Pneumocystis carinii* pneumonia (PCP).
‖ Oral candidiasis.

Findings by light microscopy closely reflected the ultrastructural changes. Mixed patterns of follicular hyperplasia and follicular involution were seen in association with degenerating FD cells at the ultrastructural level. The more severe lymphoid depletion pattern correlated with virtual loss of demonstrable FD cells and with infrequent or absent retroviral particles.

Immunoblotting was performed on sera from 20 patients at the time of lymph node biopsy.

Table 3. AIDS cases

Patients	T4/8 ratio	EIA ratio	Western blot scores						
			44	24	55	65	18	40	32
Cmu (F)*†	0·4	>13	2	4	3	3	1	2	1
Gpa‡	0·3	12	2	2	1	1	—	—	—
Dhe§	0·1	9·3	1	1	1	—	—	—	—
Kge*‖	0·1	11·4	1	3	—	—	—	—	—
Dbe*	0·04	8·5	3	—	1	1	—	—	—
Ama (F)¶**	0·3	1·8	—	1	—	—	1	—	—
Gca§††	0·11	1·9	—	1	1	1	—	—	—
Wsm§	0·5	5·8	1	1	—	—	—	—	—
	0·2	7·3	1·3	1·6	0·9	0·9	0·3	0·3	0

* Kaposi's sarcoma.
† African with lymphadenopathic Kaposi's sarcoma.
‡ CMV colitis.
§ PCP.
‖ Lymphoid interstitial pneumonia.
¶ Oesophageal candidiasis.
** Blood transfusion acquired HIV infection.
†† Cryptococcal meningitis.
(F) female.

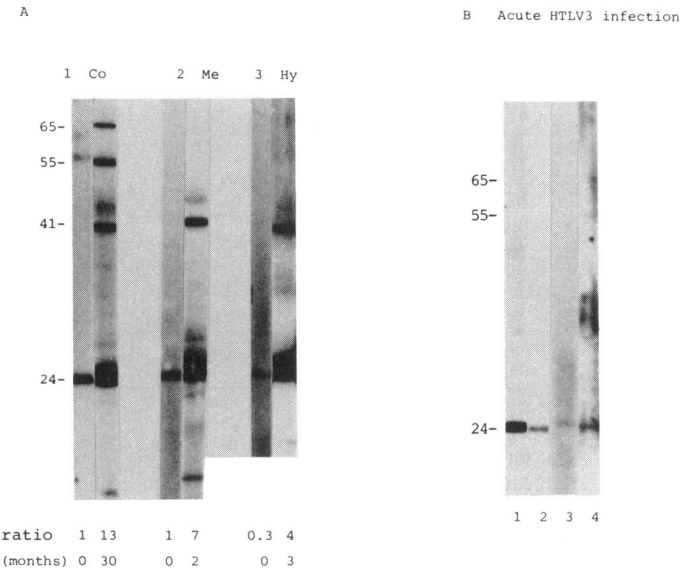

Fig. 3. (A) Three patients showing early appearance of single 24 band. Patient 1 (Jco): initial sera collected 8 weeks following multiple blood transfusion. This serum shows 24 band predominance. Subsequent sera, at time of biopsy, show multiple bands. Patients 2 (Kme) & 3 (Bhy): sera from homosexual males with asymptomatic seroconversion, showing initial 24 band and subsequent 40 and 44 bands. In all three cases initial ELISA result was borderline or negative. (B) Early positive sera from four homosexual males who presented with febrile illness associated with lymphadenopathy of less than 3 months duration. Note the presence of single 24 band in Cases 2 and 3. Cases 1 and 4 show weak 65 and 55 bands in addition to a predominant 24 band.

Table 4. Correlation of serology and pathological findings

Case	Ratio ELISA	Western Blot scores 44	24	55	65	18	40	32	Histology	FD	Retrovirus
Gts	>13	3	4	3	3	4	1	1	Hyper	+	+
Jwi	>13	4	4	2	2	2	4	1	Hyper	+	+
Jco	>13	3	4	3	3	1	3	—	Hyper	+	++B
Rwt	>13	3	3	3	3	3	2	1	Hyper	+	++
Cmw*	>13	2	4	2	2	2	3	1	Mixed	+D	++
Str	3·6	1	4	1	1	1	4	1	Mixed	+D	++
Vbe	>13	2	3	2	2	2	1	—	Hyper	+	+
Jlo	>13	1	3	1	2	1	1	—	Hyper	+	++
Jhu	7·5	3	3	2	2	—	—	—	Hyper	+	+
Nsm	4·5	2	4	1	—	2	—	—	Hyper	+	+++
Rpi	8	3	4	1	1	—	1	—	Hyper	+	+
Cdo	8·6	2	3	2	2	—	—	—	Mixed	+D	+++B
Pco	5·2	1	2	1	1	—	—	—	Hyper	+	++
Rda	7·6	2	1	2	2	—	—	—	Mixed	+	+B
Ohe*	7·2	1	1	2	2	—	—	—	Mixed	+D	+++B
Gca*	1·9	—	1	1	1	—	—	—	Mixed	+D	+++
Kge*	8·6	1	3	—	—	—	—	—	Cast	+D	+++B
Gro	5·9	1	2	—	—	—	—	—	Mixed	+D	++B
Ama*	1·8	—	1	—	—	1	—	—	Lym. dep	+D	+
Wsm*	5·8	1	1	—	—	—	—	—	Lym. dep	—	—

Hyper, Hyperplasia of follicles; Mixed, hyperplasia with some follicular involution; cast, Castleman-like changes (plasma cell variant); Lym. dep, lymphoid depletion.

FD, FD cell expansion (+). D, degeneration of FD cells present.

Quantification of retrovirus: +, occasional virions seen; ++, virions in multiple blocks; +++, abundant virions in multiple blocks; B, budding forms seen.

* AIDS cases (with Kaposi's sarcoma or *Pneumocystis carninii* pneumonia).

Comparison of the serology with the lymph node pathology reveals an inverse relationship between antibody titres and severity of ultrastructural changes. Cases showing FD cell degeneration and demonstrable budding virions had low titres of antibody (Table 4).

There was a correlation between absence of detectable p40 antibody in this group and presence of the mixed histological pattern, FD lysis and budding retrovirus ($P < 0{\cdot}05$, Fisher's exact test). Those with higher band intensity showed hyperplastic rather than degenerative follicular changes, had infrequent sequestered virions and were clinically asymptomatic apart from the lymphadenopathy.

Serology and immune function. Several parameters were analysed. This included the number of CD4 and CD8 bearing lymphocytes in blood and lymph node; 4/8 ratio, CMI assessed by Multitest (Merieux) and parameters of humoral immunity (immunoglobulin, complement and response to tetanus vaccination).

It was found that low antibody profiles by WB did not correlate with hypogammaglobulinaemia but rather occurred in association with hypergammaglobulinaemia. Anergy, as defined by a reduced DTH skin testing (partial anergy, score < 10 mm, complete anergy, 0 mm), was more frequent in groups showing lower antibody titres (i.e. lymphadenopathy and AIDS). Mean CD4 and CD4/8 were similarly reduced in these groups.

Within the group of patients with mixed follicular hyperplasia and involution and with abundant demonstrable virus, there was a reduction in mean DTH scores, peripheral blood CD4

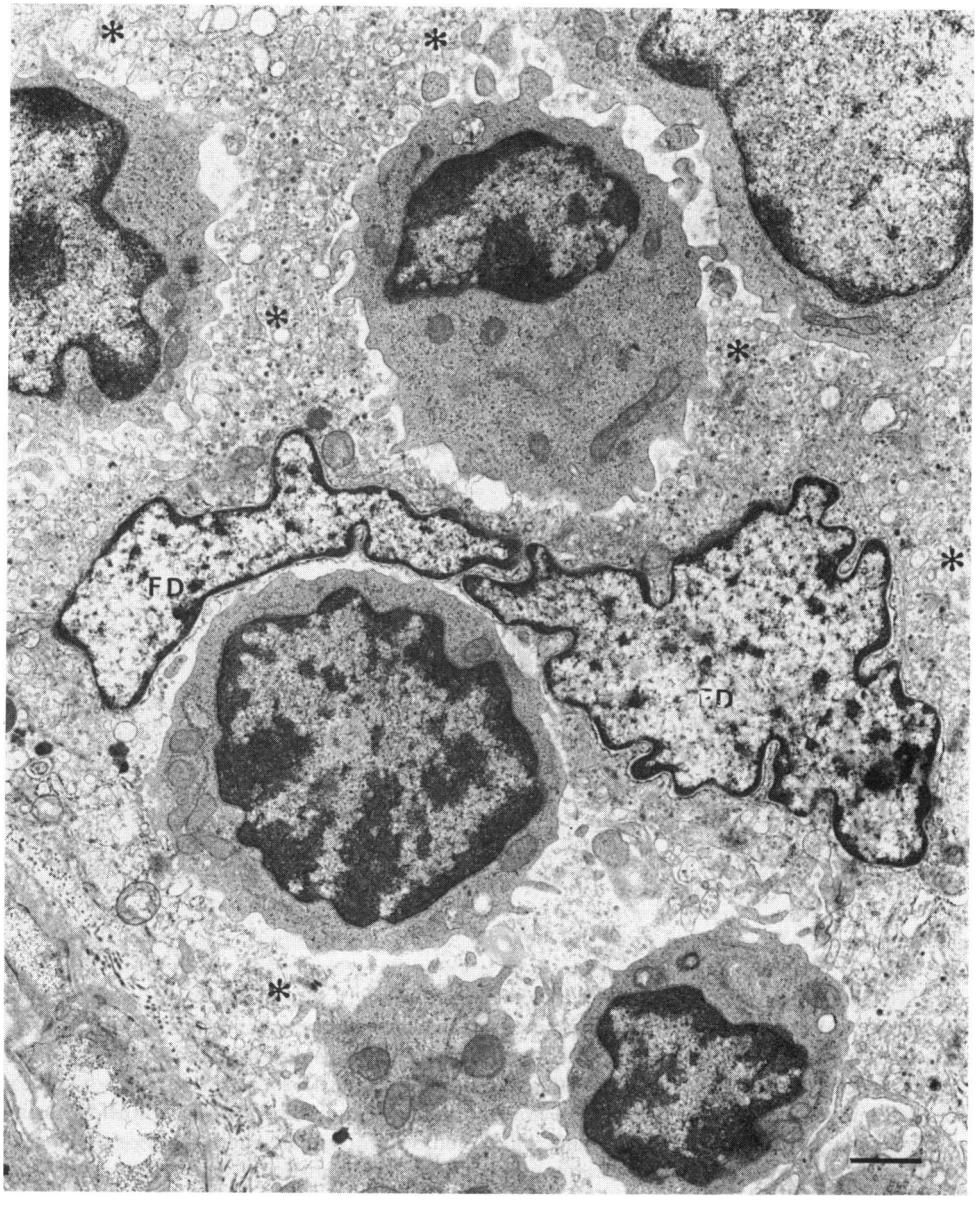

Fig. 4. Hypertrophied germinal centre follicular dendritic cell (FD), as found in nodal biopsy specimens from persistent generalized lymphadenopathy cases presenting with follicular hyperplasia. Note the distinctive crenated nucleus, and expanded spongiform cytoplasm (*) encasing adjacent lymphocytes. Electron micrograph. Bar, 1000 nm.

and CD4/8 ratios. This was associated with a reduced ELISA ratio and reduced band intensity on WB. Patients invariably had a higher CD4/8 ratio in nodes than in peripheral blood. The mean CD4/8 in blood was 0·7 compared with a lymph node CD4/8 ratio of 1·9. Two of the three patients with lymph node 4/8 ratios of less than 1·0 had lymphadenopathic Kaposi's sarcoma (Cmu & Kge, Table 3).

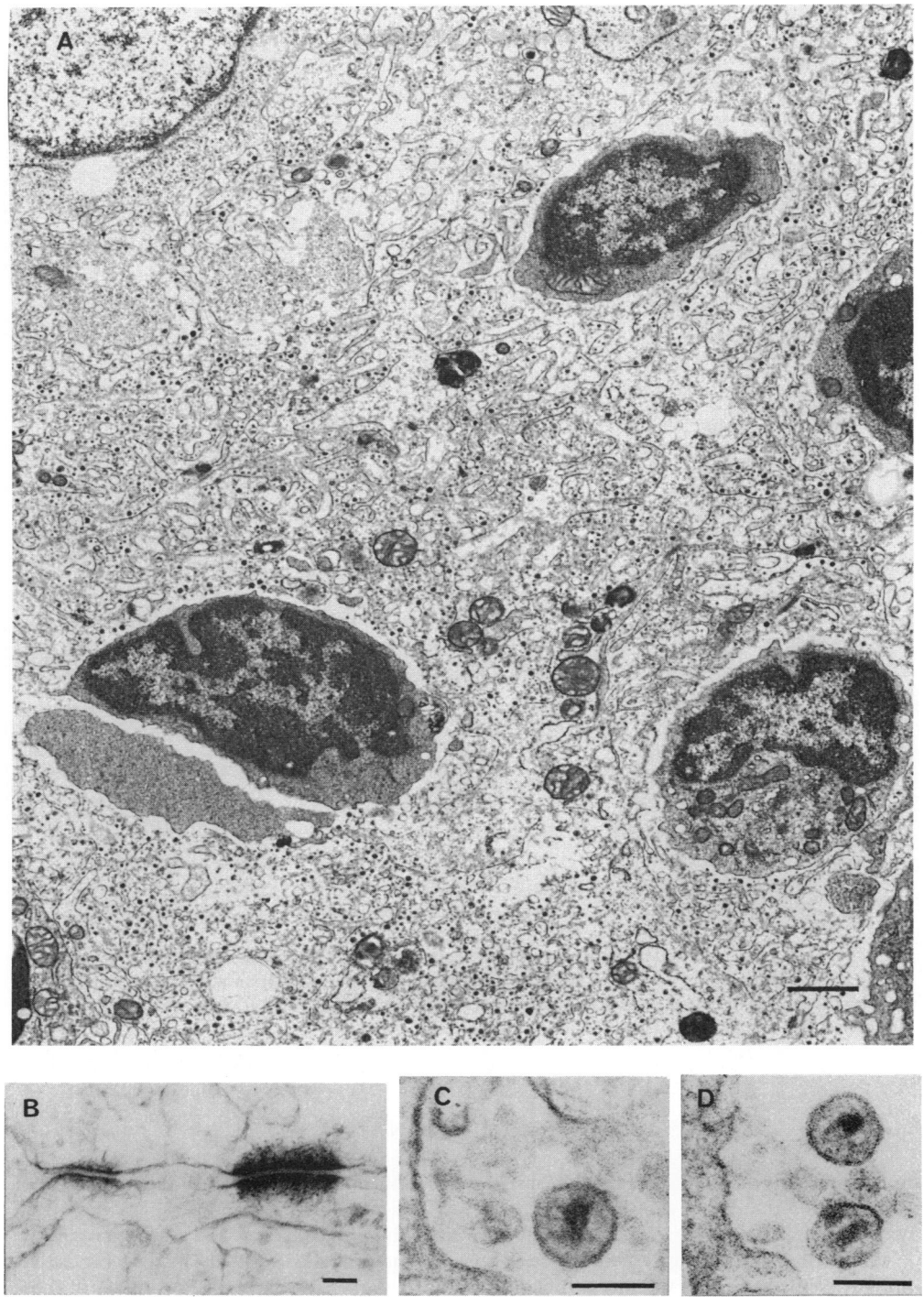

Fig. 5. Biopsied cervical node from an AIDS case. (A) Survey view of a well-developed FD cell labyrinth, with abundant sequestered 100 nm dense particles and widely separated lymphocytes. (B) Desmosomal junctions are common in the labyrinths and typify the FD cell type. (C, D) High magnifications of the individual extracellular dense particles reveal their regular lentivirus-type of retroviral morphology, including asymmetrical or discoid cores. Bars, A = 1000 nm, B–D = 100 nm.

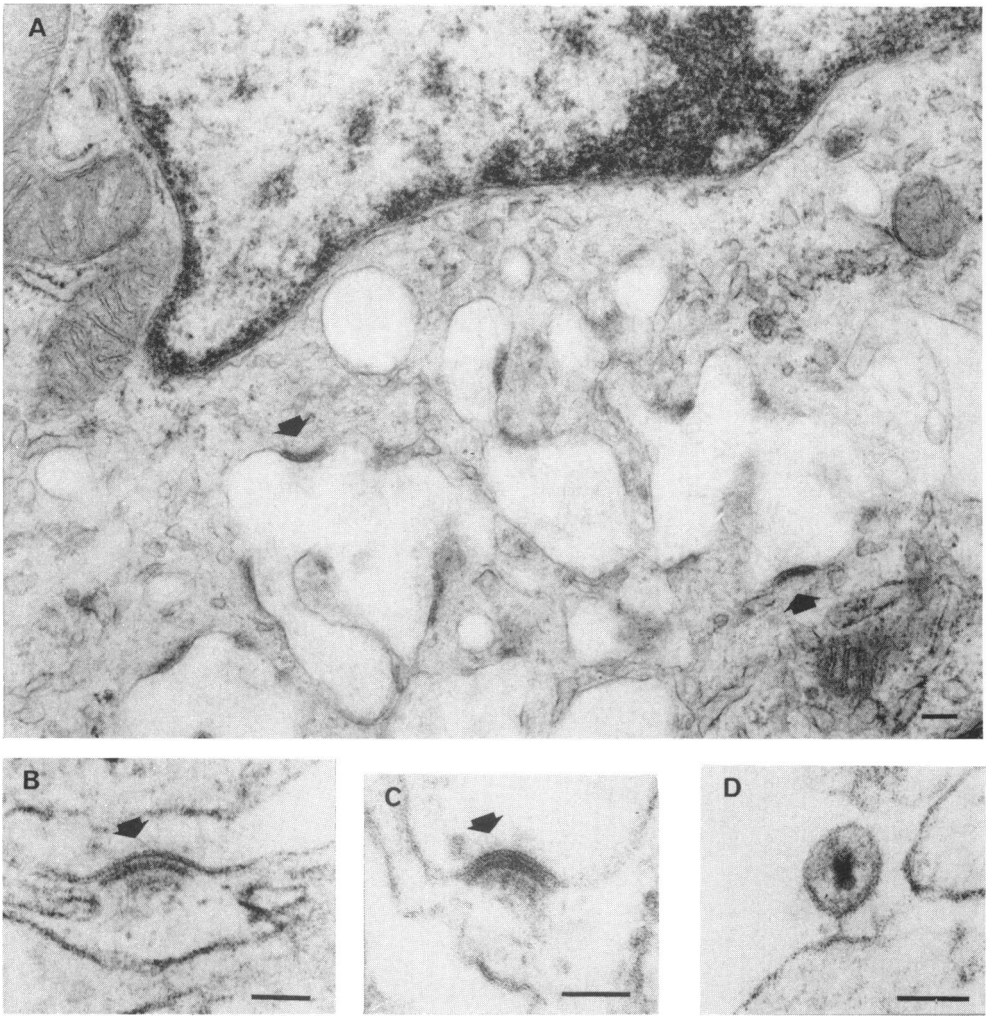

Fig. 6. (A–D) Ultrastructural detail from a germinal centre labyrinth shows budding retroviral development stages (arrows), found only on FD surface membranes and processes. In (D) an almost complete budding particle is connected to the cell process of origin by a residual thread. From an AIDS case exhibiting the mixed hyperplasia and involution pattern of follicular histology. Bar, 100 nm.

DISCUSSION

In this study we have shown that subjects with HIV infection may have very different titres of antibody and that those with lower titres tend to have more readily demonstrable virus within lymph nodes. These same subjects have more severe pathological changes and a worse prognosis.

These observations suggest that at least some of the anti-HIV antibody may be protective. Initially we believed that we may be able to demonstrate different immunoblot profiles in those subjects with more or less protective antibody. In the event, we found that the differences in profile were at least largely explicable in terms of titre rather than qualitative variations. In general, titres to individual antigens represented on the blot were predictable from the composite titre detected by the ELISA. By contrast, sera collected early in the course of infection may show a distinctive profile with a disproportionate amount of antibody to the gag antigen p24. Although our data emphasize

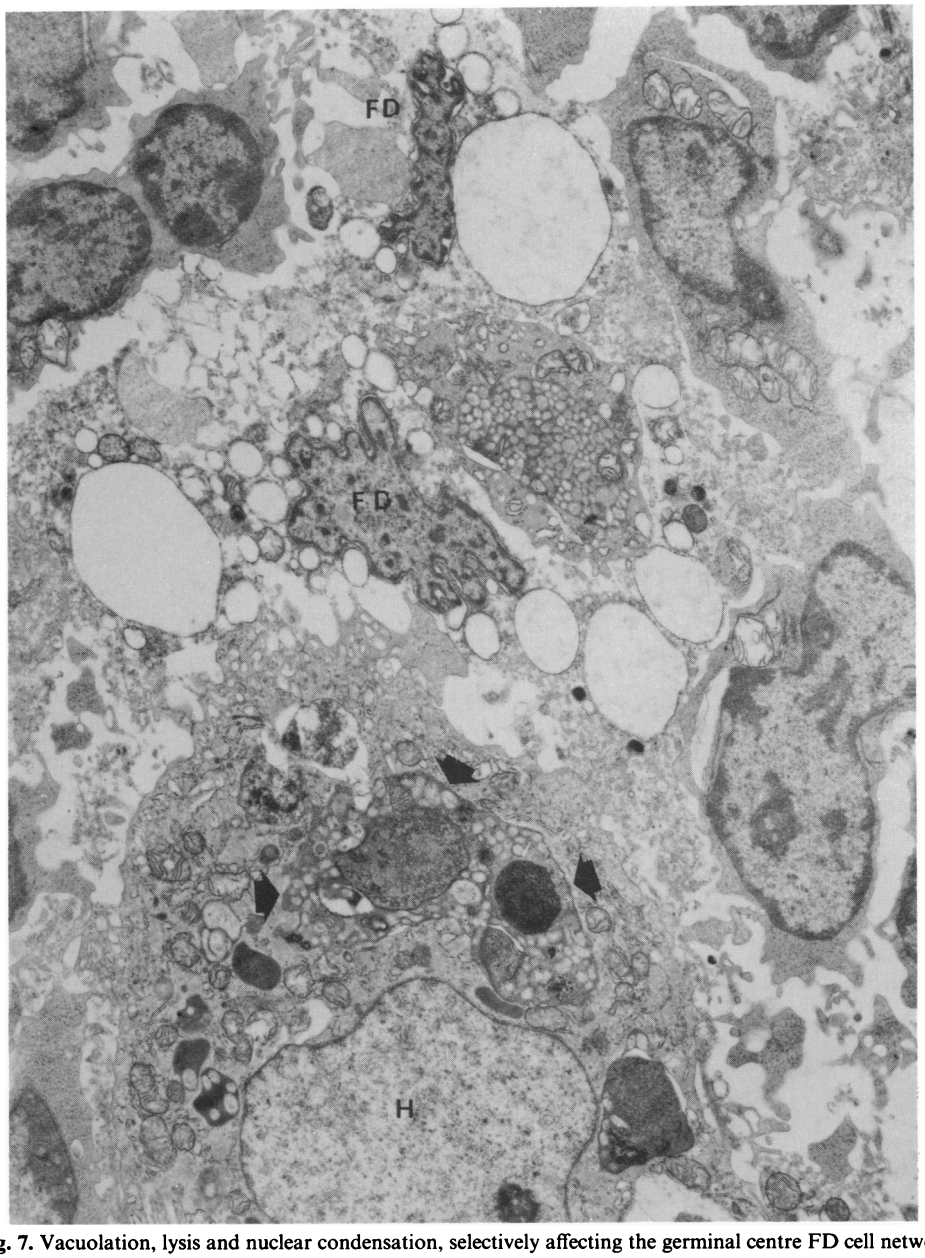

Fig. 7. Vacuolation, lysis and nuclear condensation, selectively affecting the germinal centre FD cell network; most evident in AIDS or lymphadenopathy cases with marked follicular involution. Scavenging histiocytes (H) often contain FD cell debris in their phagolysosomes (arrows). Bar, 1000 nm.

the need for quantification and standardization of immunoblotting, we conclude that this technique is unlikely to identify functionally important subpopulations of anti-HIV.

The specificity of antibodies conferring protection may be determined by functional studies. The most easily defined antibody with protective function is that defined as neutralizing antibody, i.e. antibody preventing viral infection of susceptible cells in culture (Weiss *et al.*, 1985). The precise nature and role of neutralizing antibody in HIV infection is uncertain. The envelope glycoprotein appears to bind specifically to the T4 receptor molecule (McDougal *et al.*, 1986). Antibody to an

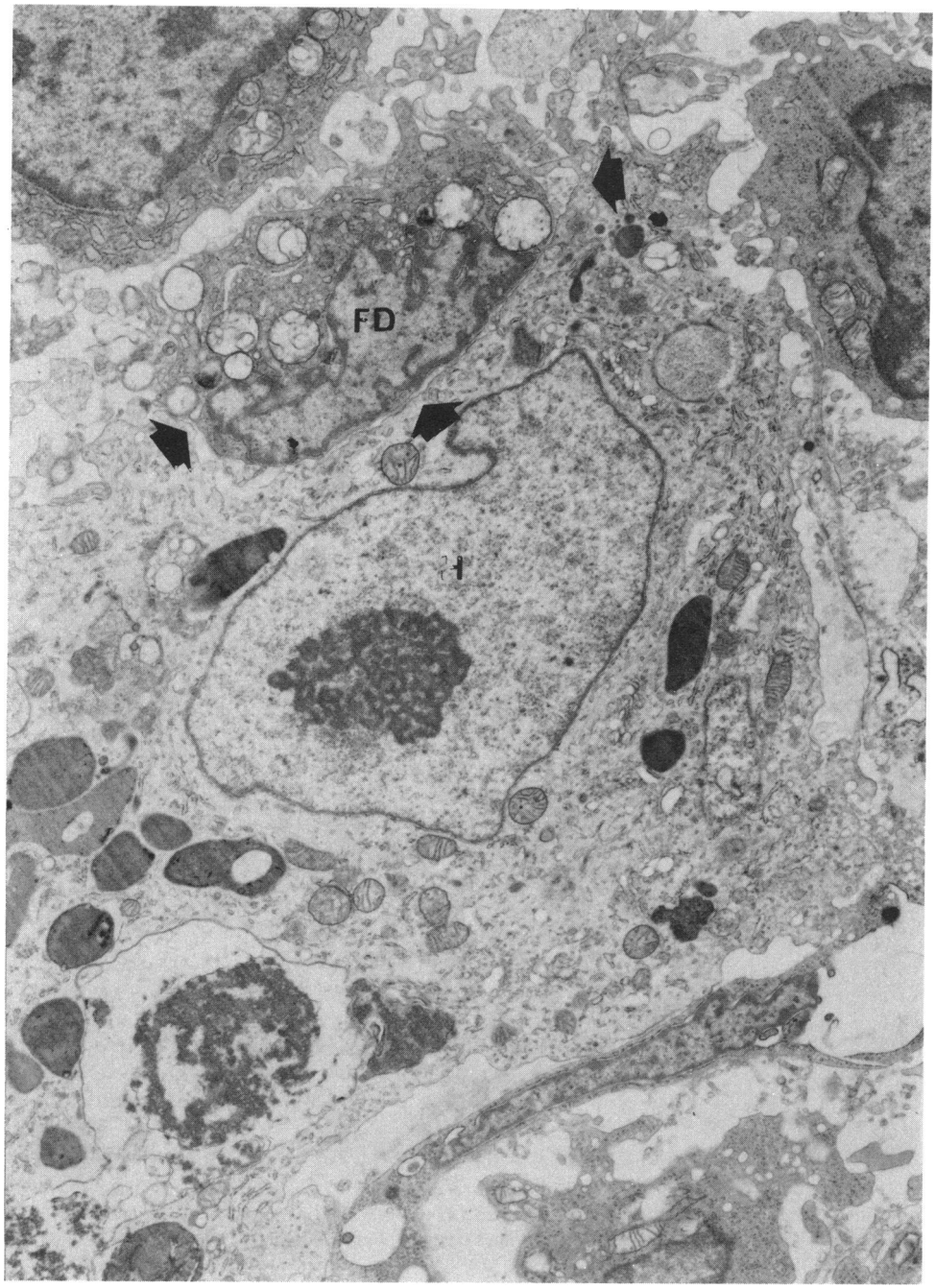

Fig. 8. Cellular profiles illustrating active ingestion of a degenerate FD cell, here partially enclosed (arrows) by a phagocytic histiocyte, or 'tingible body macrophage'. Bar, 1000 nm.

epitope involved in this binding is considered a likely candidate for antibody mediating neutralization.

The different techniques used to demonstrate neutralizing antibody appear to result in differing frequencies of these antibodies in seropositive groups. Some, (Robert-Guroff, Brown & Gallo,

1985; Weiss *et al.*, 1985) have found high titre neutralizing antibodies in only a minority of patients and particularly subjects with lesser symptoms. The presence of neutralizing antibody may be predicted by high titres using immunofluorescence or immunoblotting with gp110 under reducing conditions (Ho *et al.*, 1986). Functionally defined antibodies may be related to composite titre in the same way as specificities defined by immunoblotting. Our data suggest that protective antibodies are present in those with high titres to all viral components. It is unlikely that protective function can be assigned to a single specificity based on its absence in progressive disease (Weber *et al.*, 1986).

In view of the relationships between antibody titres, frequency of demonstrable viral particles, budding profiles and degeneration of the FD cells, it is important to reconsider the possible mechanisms for the development of immunodeficiency in HIV infection. The initial labyrinthine expansion of these cells seems likely to be a physiological phenomenon, associated with augmented antibody responses and hypergammaglobulinaemia (Armstrong *et al.*, 1985). It is now known that FD cells may express CD4, as well as Ia, Fc and C3b receptors and function as non-phagocytic antigen presenting cells within the lymph node follicles (Mandel *et al.*, 1980; Wood *et al.*, 1985; Kinet-Denoël *et al.*, 1984). With degeneration of the FD cell network, specific titres would fall and the ability to respond to new antigens would be impaired, perhaps irretrievably. Electron microscopy in clinically advanced cases shows there can be virtual ablation of the FD network with no evident reparative activity; underscoring immunohistological studies (Janossy *et al.*, 1985; Tenner-Racz *et al.*, 1986) showing clearly altered follicular dendritic patterns in patients wth AIDS. Progressive destruction of this crucial element of the germinal centre microenvironment may thus be a major factor leading to eventual loss of organized cortical follicles, as represented in the histological pattern of nodal lymphoid depletion often found in advanced AIDS cases.

The occurrence of budding virus profiles amongst the FD cells indicates that besides trapping circulating virions and immune complexes these dendritic cells can also themselves become infected and contribute to the pool of virus *in vivo*, as indeed occurs with some murine leukaemia retroviruses (Swartzendruber, Ma & Murphy, 1969). Affected FD cell networks can thus be envisaged as substantial reservoirs of trapped, developing and perhaps also degraded virus. Lymphocytes and other circulating cell types may consequently become actively or latently infected. Phagocytic histiocytes may also transport infective virus to other sites including the brain. No convincing evidence of virus developing at the surface of infected T cells has so far been revealed in our material. Furthermore, lymph node T4/8 ratios were generally maintained at higher levels than the corresponding peripheral blood ratios. The precise mechanisms of T cell dysfunction and depletion in HIV infection are still poorly understood, but functional inhibition by high concentrations of virus or immune complexes in the lymphoid micro-environment could be a contributory factor (Pahawa *et al.*, 1985). Our findings seem to argue against a concept of pathogenesis based simply on a selective and permissive retroviral infection of T4 lymphocytes, though the inability to find infected cells by electron microscopy does not exclude their occurrence at low frequency and over a long time course.

Our experience lends additional support to the case for lymph node biopsy as an aid to diagnosis (Fernandez *et al.*, 1983; Muller, Falke & Stutte, 1985), notably in seropositive cases presenting with generalized lymphadenopathy; both for exclusion of alternative pathologies, and as a potentially useful prognostic indicator. Its significance is much enhanced where facilities for experienced ultrastructural assessment are routinely available as a means of detecting retrovirus in tissues. In such centres, biopsy may be helpful in evaluating cases with low titres and indeterminate immunoblotting profiles. Further, it should be possible to monitor the germinal centre viral load and at the same time detect the onset of degenerative changes affecting the dendritic network itself.

The apparent association between low antibody titres, greater virus accumulation and more severe disease, could indicate that some individuals have a genetic inability to respond effectively to HIV infection. We have now followed patients with relatively low antibody titres on initial presentation, for up to 3 years. A tendency to further and progressive decline in antibody titres *pari passu* with clinical progression is evident. It is known that falling titres predict the development of AIDS (Biggar, Melbye & Ebbesen, 1985; Lange *et al.*, 1985); in some cases this may be a consequence of loss of FD cells but in these and other cases the immune response may be inherently defective.

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