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Cellular Composition of Germinal Centers in Lymph Nodes after HIV-1 Infection: Evidence for an Inadequate Support of Germinal Center B Lymphocytes by Follicular Dendritic Cells

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Germinal centers in lymph nodes with follicular hyperplasia from 15 patients with HIV-1 infection were analyzed by qualitative and quantitative electron microscopical methods and compared with control follicular hyperplasia (FH). Using a pattern recognition method, two main clusters were recognized within the germinal centers of HIV and FH lymph nodes on the basis of the relative frequencies of small centroblast and centrocytes. All FH lymph nodes and 6 HIV-1 lymph nodes (HIV-Clu-1) were placed in cluster 1; 9 HIV-1 lymph nodes (HIV-Clu-2) formed cluster 2. Germinal centers in the HIV-Clu-2 lymph nodes were characterized by a cell composition of predominantly lymphoid blasts and decreased numbers of centrocytes, but without altered numbers of mitotic figures. The frequency distribution of ultrastructurally distinct FDC subtypes differed between these clusters. In HIV-Clu-2 the frequencies of FDC types with an undifferentiated and regressive morphology occurred at a higher frequency, whereas FDC types with a highly differentiated morphology had a lower frequency. We conclude that 9 out of 15 lymph nodes with HIV-1 associated follicular hyperplasia show changes in FDC morphology indicative of a less differentiated functional stage of FDC. The changes in FDC morphology are closely associated with changes in the germinal center B-cell population resulting in an inverted blast to the centrocyte ratio. © 1992 Academic Press, Inc.

INTRODUCTION

Infection with HIV-1 virus can be accompanied by the development of persistent generalized lymphadenopathy (PGL). Several patterns of histopathological changes have been described in PGL (1–6). The most common feature is a florid follicular hyperplasia (FH). In this condition, regressive changes, such as loss of mantle zones and follicular transformation and fragmentation, can be observed. Immunohistochemical studies have shown the disintegration or fragmentation of the reticulum of follicular dendritic cells (FDC) (2, 7, 8). These histological changes, segregated or in combination, are related to the clinical stage of the HIV-1 infection (9).

The (immuno)histopathological features are not pathognomonic for HIV-1 infection (10–14). Disintegration of the framework of follicular dendritic cells may occur in other conditions of follicular hyperplasia (11). It cannot be excluded that similar morphological appearances of germinal centers in both AIDS-related PGL and non-AIDS-related FH are the result of different pathologic mechanisms (13). For FDC, literature data (15–20) suggest a cytopathic action of the HIV-1 virus or viral proteins present within the immune complexes on the plasma membrane of FDC. These studies provide evidence that FDC are susceptible to HIV-1 infection and produce virus. Since FDC function as accessory cells (21), such cytopathic effects on FDC may interfere with germinal center B-cell processing.

Thus far, no ultrastructural study of the germinal center compartment after HIV-1 infection is available, which includes the simultaneous analysis of B cells and FDC. Ultrastructurally, distinct B-cell subsets can be distinguished in germinal centers (22). Similarly, seven types of FDC are recognized on the basis of nuclear and cytoplasmic features and cell surface characteristics. A detailed analysis of B-cell and FDC subtypes in germinal centers of the tonsil (23) indicates that these FDC subtypes represent distinct differentiation stages of FDC. In the present study we applied this expertise to germinal centers in lymph nodes of HIV-1-infected patients. These findings were related to the histopathological and immunohistological observations in the HIV-1 related lymphoadenopathy and clinical stage of the patients.

MATERIAL AND METHODS

Lymph Nodes

Fresh lymph node biopsies were processed for multiple studies. The protocol included routine histology, enzyme- and immunohistochemistry, and electron microscopy (24). Fifteen lymph nodes (HIV1–HIV15) of HIV-1-infected patients were studied. On the basis of

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conventional (immuno)histopathology (15), all these cases were categorized as stage 1 (follicular hyperplasia) (2). Clinical staging of the patients was done according to CDC criteria (25).

A control group of eight lymph nodes (FH1–FH8) with follicular hyperplasia was selected from our files on the basis of the histpathological diagnosis. These biopsies were taken from patients with lymphoadenopathy on suspect of malignancy. Lymph nodes originating from patients having any clinical or serological evidence of viral infection or immunologic disease were excluded.

Morphometry

Cell composition. None of the germinal centers showed any polarity by (immuno)histopathology. The following types of germinal center cells (22, 23) were identified on the basis of their ultrastructure and were quantified on low power electron micrographs: cleaved blast (ClB), immunoblast (IB), large (>12 μm) centroblast (CBL), small (<12 μm) centroblast (CBS), centrocyte (CC), centroplasmacytoid cell (CPC), lymphocyte (LYC), multilobated cell (MLC), histiocytic reticulum cell (HRC), and follicular dendritic cell (FDC). For the determination of the relative frequency of these cell types, three follicular structures were selected by light microscopy in 1-μm sections. A set of six electron micrographs (magnification 4000×; representing a total area of 14,000 μm^2) was randomly taken from each follicular structure (22) in the corresponding ultrathin section. Mantle zones were not included in this analysis.

Follicular dendritic cells. In each biopsy, ultrastructural morphometry was performed on the three germinal centers mentioned above. The number of FDC per surface area and their relative volume was determined on six electron micrographs (magnification 4000×) of each germinal center. The relative volume of FDC cell bodies, as well as their nuclei, was measured by a point counting method (test grid lattice, 5 μm) as described previously (24). From these data the mean nucleus/cytoplasm ratio was calculated.

Seven ultrastructurally distinct types of FDC (23) were distinguished: FDC.1, primitive; FDC.2, undifferentiated; FDC.3, intermediate; FDC.4, differentiated; FDC.5, secretory; FDC.6, regressive, pale type; and FDC.7, regressive, dark type. The main characteristics of these FDC types are outlined in Table 1. The relative frequency of the various types of FDC was assessed in the same germinal centers by scanning the ultrathin sections directly in the electron microscope. The incidence of FDC containing distended cisterns of rough endoplasmic reticulum (RER) and lysosomes was concomitantly recorded. For this purpose 56–151 FDC per biopsy were graded. If necessary, additional sections at another section level of the same germinal centers were analyzed.

Data Analysis

Cluster analysis. The data on the relative frequencies of the lymphoid and nonlymphoid cell types in the germinal centers of the lymph node biopsies were analyzed by nonsupervised pattern recognition methods and supervised statistical evaluation.

TABLE 1

Summary of the Main Features of FDC Subtypes in Lymph Nodes of HIV-1-Infected and Non-HIV-1-Infected Patients

FDC subtype	Denotation	Ultrastructure
FDC.1	Primitive	Very scarce cell organelles; when present, poorly developed. Filamentous cytoplasmic matrix. No villous extensions or dense deposits.
FDC.2	Undifferentiated	Stellate cells with few organelles; polyribosomes. Submembranous intermediate filaments. No villous extensions covered with dense deposits.
FDC.3	Intermediate	Rounded cells with moderately developed rough endoplasmic reticulum (RER) and inconspicuous Golgi area. Some plumb cytoplasmic extensions having no dense deposits. Submembranous filament condensations.
FDC.4	Differentiated	Rounded cells with well-developed RER and Golgi systems. Some submembranous filament condensations. Tiny villous plasma membrane protrusions with dense (immune complex) deposits.
FDC.5	Secretory	Like FDC.4. But RER, Golgi area, and villous plasma membrane web extremely developed.
FDC.6	Regressive, pale	Elongated cells with large proportions of cytoplasm. Both RER (dilated) and Golgi area inconspicuous. Lysosomes. Moderate villous extensions with dense (immune complex) deposits.
FDC.7	Regressive, dark	Stellate cells with electron-dense cytoplasmic and nuclear matrix. Dilated RER; inconspicuous Golgi area; clusters of free ribosomes. Villous extensions and dense deposits not regularly present.

The complete dataset of the relative frequencies of individual types of germinal center cells was subjected to a computerized cluster analysis using the Biopat method (26). Cluster analysis is a technique that recognizes the most striking pattern in a set of data. The method is nonsupervised because the formation of clusters is achieved without intervention of the investigator. The grouping of objects (in the present study, lymph node biopsies) is solely based on the properties of the objects (in this analysis the data on relative frequencies of all distinct cell types). The details of this technique are explained by Sneath and Sokal (27). In short, the method repeatedly combines the two objects that are the most similar into a new object. Two objects are considered to be similar if they show little dissimilarity in the frequencies of the germinal center cell types. The degree of dissimilarity is expressed as a "mean city block distance" (28) and is based on the summation of the differences between two objects with respect to all of their properties (frequencies of all cell types). The replacement of any pair of similar objects by a new combined one depends on the cluster criterion. In this study we used Ward's criterion (29) for the generation of clusters.

Further analysis of the data on both germinal center cells and FDC of the thus defined groups was performed with the (supervised) nonparametric Mann–Whitney U test. All parameters on germinal centers

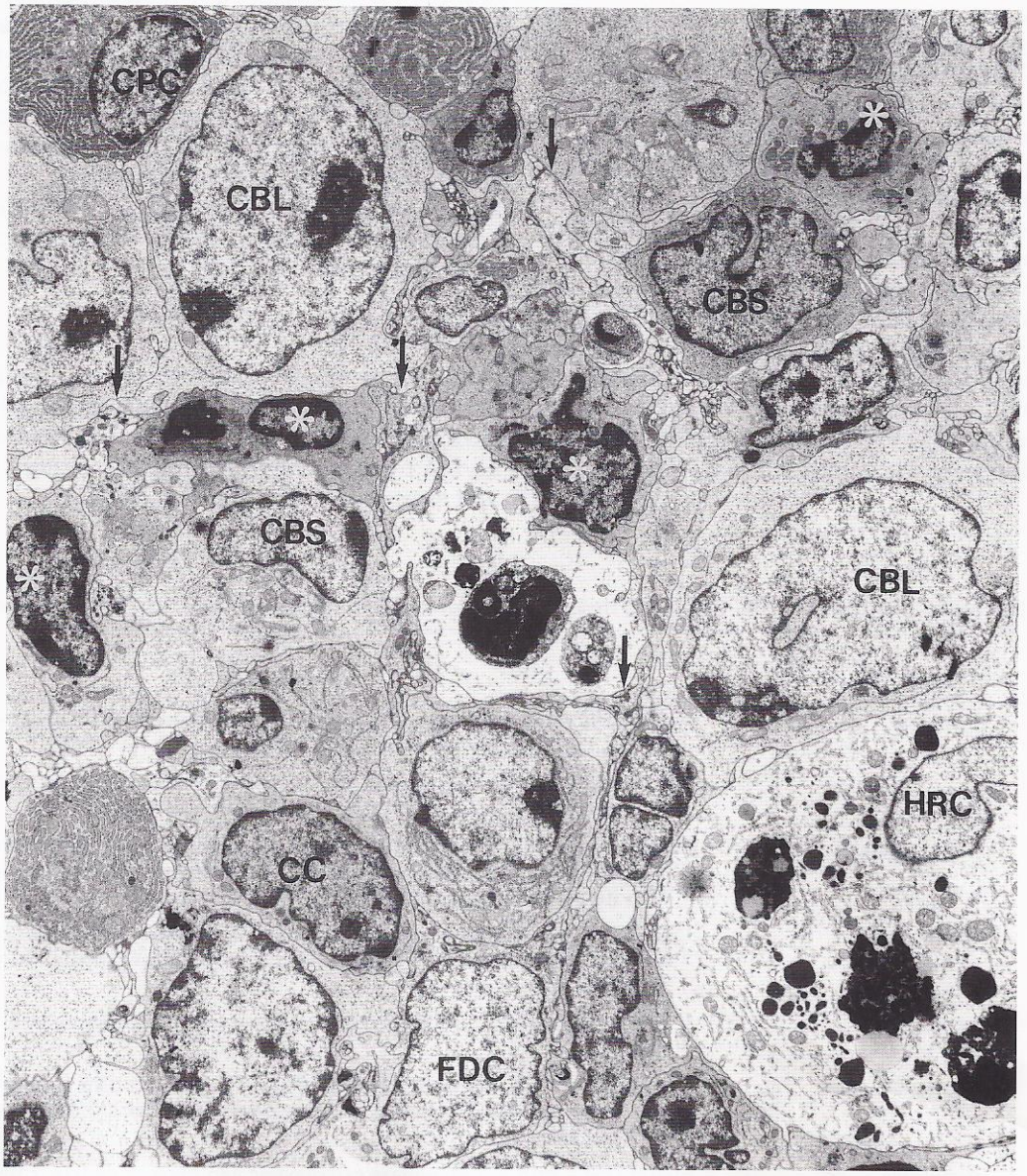

FIG. 1. Electron micrograph of a germinal center (control group, biopsy FH8) showing the various types of germinal center cells within the web of extensions (arrows) of follicular dendritic cells (FDC). HRC, histiocytic reticulum cell; CBL, large centroblast; CBS, small centroblast; CC, centrocyte; CPC, centroplasmacytoid cell. Asterisks: scattered small lymphocytes with a heterochromatic nucleus; the cytoplasm may contain electron-dense granules (arrowheads). ×4000.

were subjected to a rank correlation test. Both procedures were applied at a probability level of 5%.

RESULTS

Cell Composition of Germinal Centers

Germinal center cells. Ultrastructurally, a spectrum of germinal center cell types is identified. Some of these cell types are illustrated in Fig. 1. Similar to germinal centers of the FH group, the majority of these cell types (except immunoblasts and multilobated cells) were recognized in lymph nodes of the HIV-1-infected individuals (Fig. 2). By light microscopy on semithin 1-μm sections and by electron microscopy, most of the germinal centers consisted of an intermingled mixture of germinal center cells. Some (six) of the HIV-1 lymph nodes showed small accumulations of centroblasts dispersed in the germinal centers. The absence of a polarity (as judged by light and electron microscopy) in these germinal centers allowed a quantitative determination of the cell composition. The relative frequencies of cell types as well as the total blast frequency (TBL: the summation frequencies of immunoblasts, cleaved blasts, and small and large centroblasts), incidence of mitosis (MIT), blast/centrocyte ratio (BL/CC ratio), and size of the cell sample (analyzed in an area of 42,000 μm^2, obtained from three germinal centers) are given in Fig. 3. The HIV lymph nodes showed a large variation in the frequencies of cleaved

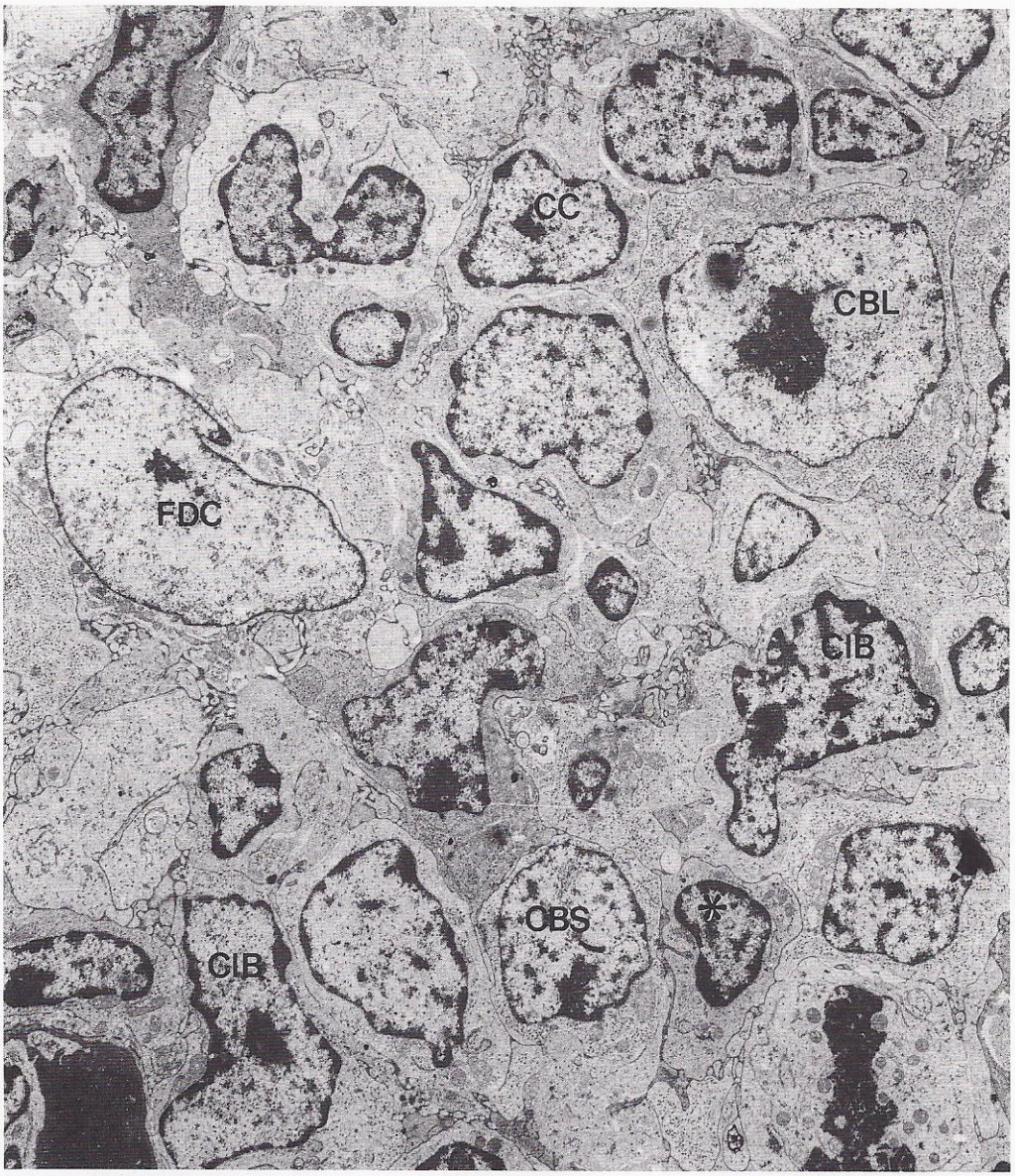

FIG. 2. Low power electron micrograph of a germinal center of a lymph node from a HIV-1-infected patient (biopsy HIV6). Follicular dendritic cell (FDC) within a population of cleaved blasts (ClB), large (CBL) and small (CBS) centroblasts, and centrocytes (CC). Note the predominance of blasts. ×4000.

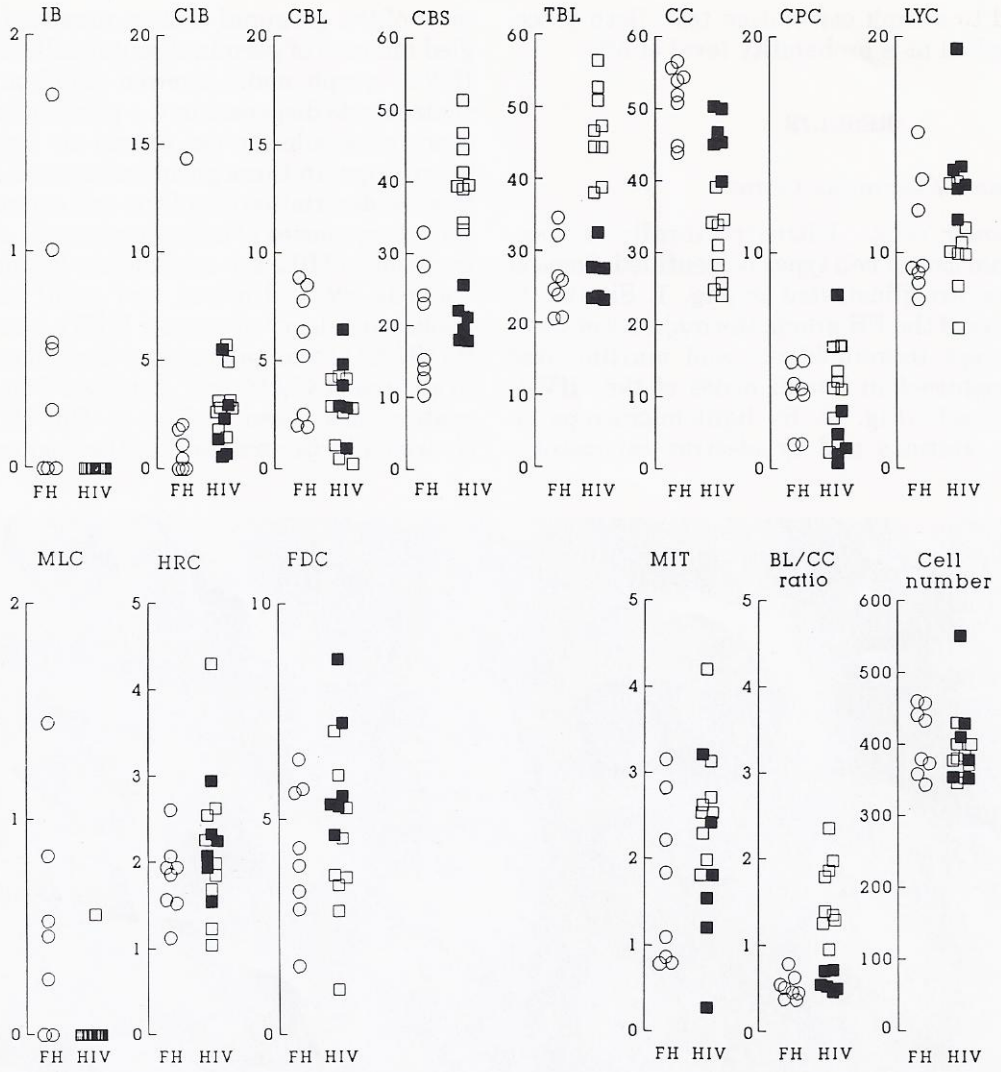

FIG. 3. Relative frequencies of the various parameters in germinal centers determined in hyperplastic lymph nodes of HIV-1-infected (HIV) and non-HIV-1-infected patients (FH). For abbreviations, see Table 1. Subgroups of the HIV-1-infected patients, as revealed by cluster analysis, are indicated by open (HIV-Clu-1) and closed (HIV-Clu-2) symbols.

blasts, small centroblasts, total blasts, centrocytes, follicular dendritic cells, and blast/centrocyte ratio.

Cluster analysis. The relative frequencies of the lymphoid and nonlymphoid cell types in the germinal centers of the 23 lymph nodes were subjected to cluster analysis. This yielded two main clusters (Clu-1 and Clu-2). The main discriminating parameter proved to be the small centroblast. In the lymph nodes belonging to Clu-1, this parameter is always lower than 30%, whereas it had values higher than 30% in all cases of Clu-2 (see also Fig. 3). Lymph nodes HIV1–HIV5 and HIV8 appeared together with all control FH lymph nodes in Clu-1, whereas the remaining 9 lymph nodes of HIV-1-infected patients formed Clu-2. These two groups of HIV lymph nodes are further referred to as HIV-Clu-1 and HIV-Clu-2, respectively.

Statistical analysis. The values of the individual parameters of the three thus defined subgroups (FH, HIV-Clu-1, and HIV-Clu-2) are given in Table 2. There were no statistical differences between FH and HIV-Clu-1 lymph nodes for all parameters tested. Compared to FH and HIV-Clu-1, germinal centers of HIV-Clu-2 lymph nodes showed an increased frequency of cleaved blasts and small centroblasts, and a decreased frequency of centrocytes. This resulted in a higher blast/centrocyte ratio. The incidence of mitotic figures did not differ among the three groups. The HIV lymph nodes of Clu-1 and Clu-2 showed additional significant differences in the relative frequencies of FDC and lymphocytes.

Follicular Dendritic Cells

Morphological aspects of FDC. Follicular dendritic cells are recognized in germinal centers as electron-lucent, often binucleate cells with elaborate cytoplas-

TABLE 2

Ultrastructural, Immunohistochemical Data on Lymphoid Germinal Center Cells and Follicular Dendritic Cells Present in Germinal Centers of Hyperplastic Lymph Nodes and Clinical Data of HIV-1-Infected Patients (HIV-Clu-1 and HIV-Clu-2) and Non-HIV-Infected Patients (FH)

	FH		HIV-Clu-1		HIV-Clu-2	
	Mean	SD	Mean	SD	Mean	SD
Ultrastructural characteristics	$n = 8$		$n = 6$		$n = 9$	
Immunoblast (IB)	0.5	0.6	0	0	0	0
Cleaved blast (ClB)	2.5a	1.6	2.2	2.7	3.2a	1.3
Large centroblast (CBL)	5.4	2.7	3.7	0.7	2.6	1.5
Small centroblast (CBS)	17.6a	6.0	20.7b	1.7	41.1ab	5.5
Blast (total, TBL)	26.2a	4.7	26.4b	3.3	46.8ab	5.7
Centrocyte (CC)	51.5a	4.5	46.1b	3.7	30.9ab	5.1
Centroplasmacytoid cell (CPC)	3.3	1.2	3.2	2.7	2.9	1.7
Lymphocyte (LYC)	10.6	2.5	13.9a	2.8	10.5a	2.0
Multilobated cell (MLC)	0.5	0.4	0	0	0.6	0.2
Histiocytic reticulum cell (HRC)	1.8	0.4	2.2	0.4	2.2	0.9
Follicular dendritic cell (FDC)	4.2	1.5	6.1a	1.4	4.2a	1.7
Ratio blast/centrocyte (BL/CC ratio)	0.52a	0.13	0.58b	0.10	1.60ab	0.40
Mitotic figures (MIT)	1.7	0.9	1.8	0.9	2.7	0.7
Total cell number	407	44	418	65	381	25
Presence of virus-like particles						
35-nm particles	0		3		6	
80- to 120-nm particles	0		6		7	
Histopathological grading						
Germinal centers						
Enlarged germinal centers	4		6		8	
Mantle zone effacement	4		6		6	
Nude germinal centers	2		4		5	
Immunohistology						
FDC meshwork	$n = 8$		$n = 3$		$n = 9$	
Indentation (DRC-1 antibody)	nda[b]		0		3	
Minor fragmentation (DRC-1 antibody)	nda		1		4	
Major fragmentation (DRC-1 antibody)	nda		0		2	
Follicular localization of HIV-1 p15	nda		3		6	
Follicular localization of HIV-1 p24	nda		3		7	
Clinical stage[a]	—		$n = 6$		$n = 9$	
Stage III	—		4		6	
Stage IV	—		2		3	
Duration of disease (months)	nda		7–20		1–56	

Note. n, number of biopsies; SD, standard deviation. Corresponding characters (a or b) behind one pair of data in a row indicate significant differences (U test; $P \leq 0.05$).

[a] Clinical stage according to CDC criteria (25): stage III, persistent generalized lymphadenopathy; stage IVa, constitutional disease.

[b] No data available.

mic extensions enclosing the lymphoid cells and forming the meshwork of the germinal center (Figs. 1 and 2). The FDC meshwork is widened in germinal centers belonging to the HIV-Clu-2 group. In these areas, larger groups of blast cells are present between the extensions of FDC.

In the lymph nodes of the present study, the seven types of FDC described previously in tonsil germinal centers were also distinguished (Table 1). FDC.4 and FDC.5 in these lymph nodes are illustrated in Figs. 4a and 4b. FDC.7 (Fig. 5a) is present in a much higher frequency in germinal centers of the lymph nodes than in tonsils (23). FDC.7 is observed around capillaries and in larger groups of cells, interconnected by desmosomal contacts with other FDC types (Fig. 5b).

In all FDC types, swollen RER cisterns may occur. They are the most frequent in FDC.6 and FDC.7. Small lysosomal structures of moderate electron density have a similar distribution in these FDC types. They often have a lamellar substructure (Fig. 5c).

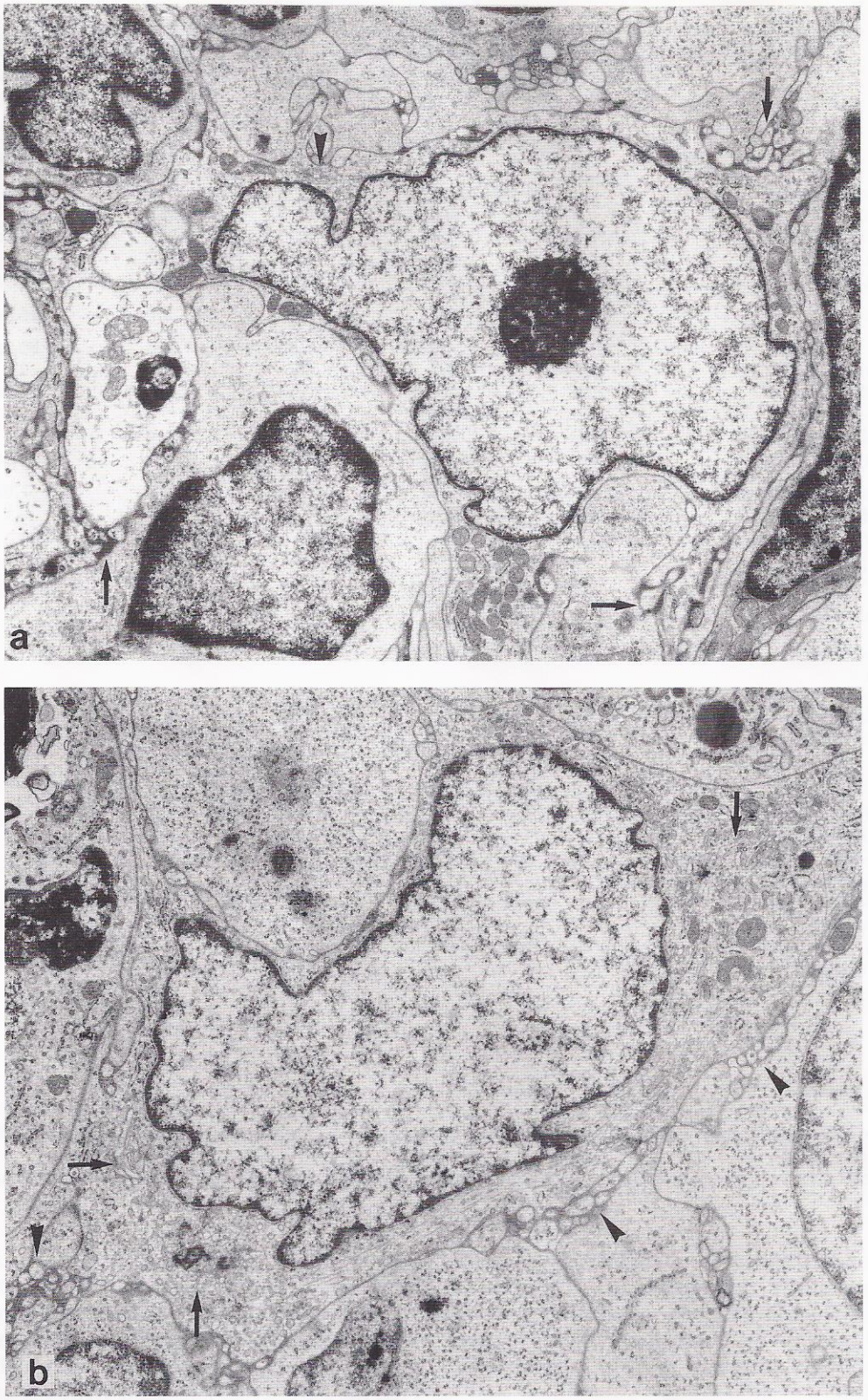

FIG. 4. (a) FDC.4, HIV-1 lymph node; FDC with well-developed cell organelles. Numerous villous extensions with extracellular electron-dense depositions are present (arrows). Arrowhead: Golgi complex. (b) HIV-1 lymph node; FDC.5 with a relatively large amount of cytoplasm, containing elongated cisterns of endoplasmic reticulum and several Golgi complexes (arrows). Golgi-associated vesicles are numerous. Electron-dense depositions between villous extensions are numerous (arrowheads).

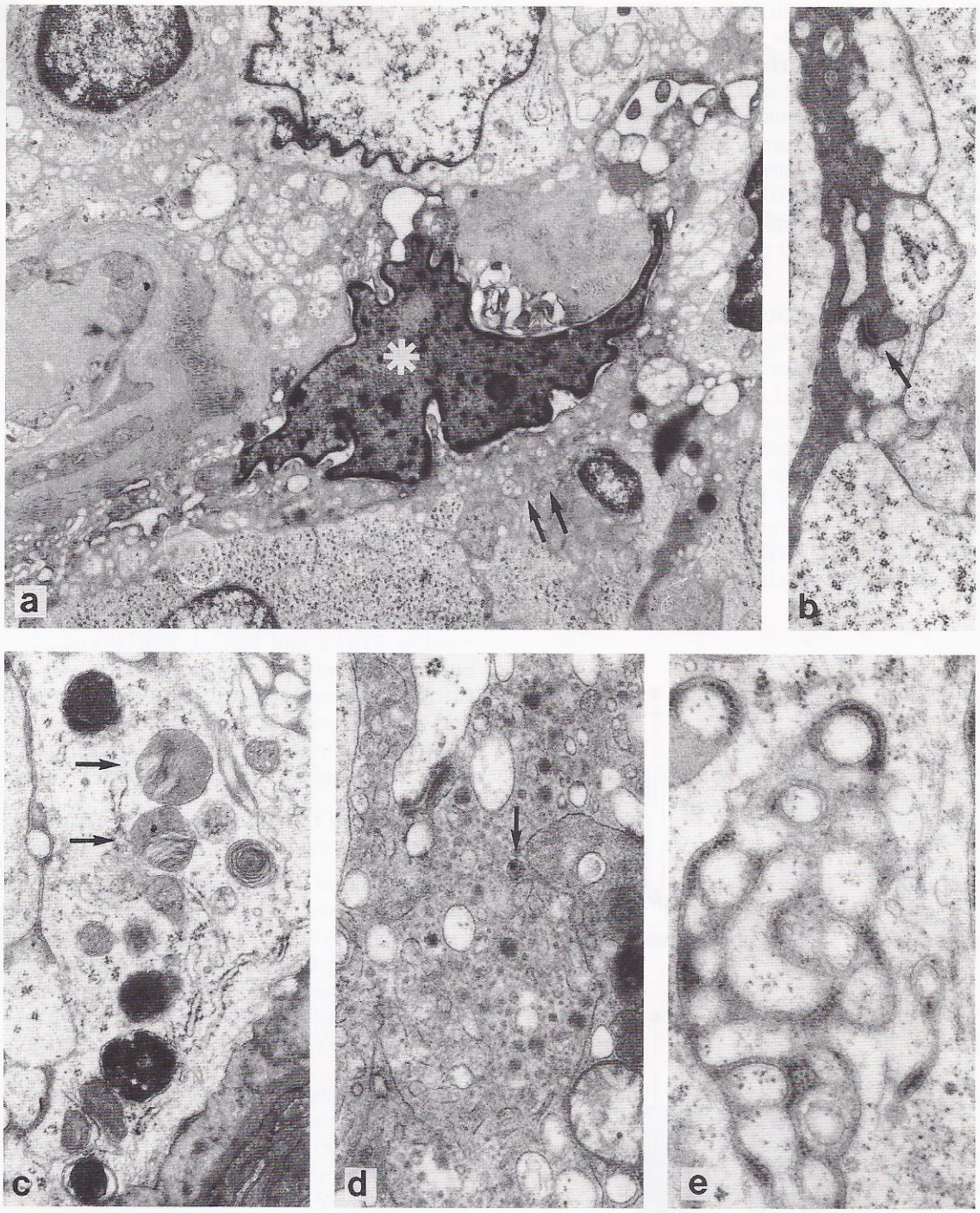

FIG. 5. (a) FDC.7; "dark" type (*). The cytoplasmic matrix as well as the heterochromatic nucleus have a high electron density. Swollen mitochondria and dilated cisterns of endoplasmic reticulum are generally present. Cytoplasmic extensions are elongated and microvillous projections (arrows) are present. ×9200 (b) Desmosomal connection of a cytoplasmic extension of FDC.7 with an extension of a FDC type with an electron-transparent appearance (arrow). ×32,000. (c) Accumulation of lysosomal structures in the cytoplasm of FDC. Some of these structures have a lamellar substructure (arrow). ×32,000. (d) Virus-like particles between extensions of FDC. Some of them have morphological features of HIV-1 virions (arrow). ×32,000. (e) small 35-nm particles lined up within the electron-dense depositions present at the cell surface between villous extension of FDC. ×32,000.

Electron-dense deposits, probably representing immune complex depositions, were present between the villous extensions of FDC throughout the germinal center, but the density of these deposits varied for the individual FDC types. Virus-like particles measuring 80–120 nm in diameter (Fig. 5d) were observed between the extensions of FDC in 13 out of 15 lymph nodes with HIV-1 infection. Most of these virus-like

particles contained a large electron-dense core; few had an ultrastructure compatible with HIV-1 virions. Electron-dense particles measuring about 35 nm in diameter (Fig. 5e) were observed in 9 biopsies of HIV-1 positive individuals. Both forms of particles were exclusively located extracellularly between the villous extensions of FDC. They were present in lymph nodes of both the HIV-Clu-1 and HIV-Clu-2 groups, and were not observed in the FH lymph nodes (Table 2). We did not observe budding of viral particles in any germinal center cell.

Quantitative aspects of FDC. The data on FDC distribution are presented in Fig. 6a. The number of FDC per surface unit did not differ among the germinal centers of the FH, HIV-Clu-1, and HIV-Clu-2 lymph nodes. The relative volume of the FDC was higher in the HIV-Clu-1 germinal centers than in the FH group. The nucleus/cytoplasm ratio of FDC is decreased in both the HIV-Clu-1 and HIV-Clu-2 lymph nodes. Since the number of nuclei and the relative volume of FDC nuclei do not differ after HIV-1 infection (data not shown), the decreased nucleus/cytoplasmic ratio is mainly caused by an increase of the cytoplasmic volume.

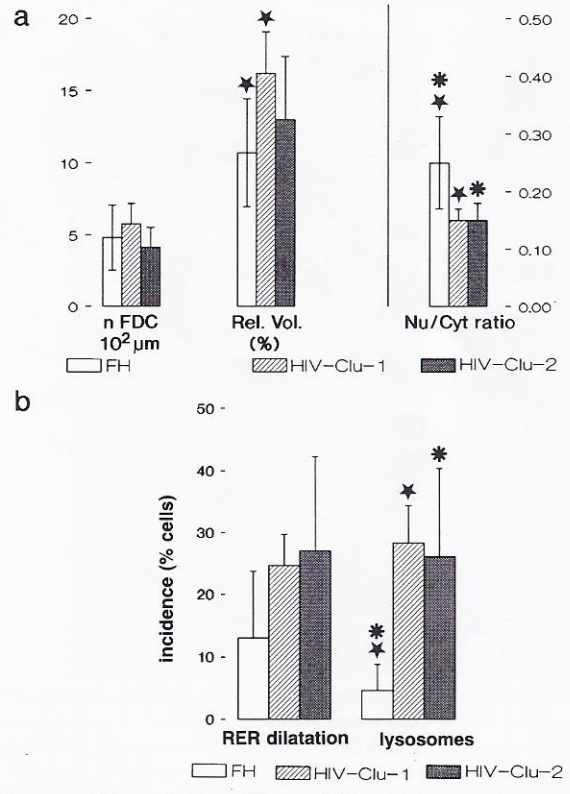

FIG. 6. (a) Quantitative data on FDC distribution in germinal centers in lymph nodes from HIV-1-infected (HIV-Clu-1 and HIV-Clu-2) and non-HIV-1-infected patients (FH). n FDC, number of FDC; rel. vol., relative volume of germinal center occupied by FDC; Nu/Cyt ratio, ratio of nuclear and cytoplasmic volume. (b) Quantitative data on the incidence of RER dilatation and lysosomes in FDC in germinal centers of HIV-1-infected (HIV-Clu-1 and HIV-Clu-2) and non-HIV-1-infected patients (FH). Corresponding symbols (★ or *) on top of the columns representing a distinct parameter indicate significantly different pairs ($P \leq 0.05$).

The data on RER and lysosomes are summarized in Fig. 6b. The percentage of cells containing RER dilatation tended to be higher in HIV-Clu-1 and HIV-Clu-2 lymph nodes than in FH cases. When all the HIV-1 lymph nodes were considered as one group, the difference from the FH cases was significant. The incidence of FDC containing lysosomal structures is higher in both groups of HIV lymph nodes than in FH lymph nodes.

The data on relative frequencies of the FDC types are depicted in Fig. 7. The frequency distribution of FDC types was similar in both the FH and HIV-Clu-1 groups. In the HIV-Clu-2 group, the relative frequencies of FDC.4 and FDC.5 were lower than in the FH group. Reversely, the relative frequencies of FDC.6 and FDC.7 were higher.

The results of the analysis of covariance between the data on FDC and those on some other parameters of these germinal centers are given in Table 3. This analysis showed a relationship with the occurrence of FDC types and certain B-cell characteristics of the germinal center, e.g., mitosis and blast/centrocyte ratio. In contrast to FDC.4 and FDC.5, FDC.6 and FDC.7 showed an opposite relationship with the blast/centrocyte ratio. Also, regressive cytological changes (RER swelling and the presence of lysosomes), which are increased after HIV-1 infection, showed a relation with the relative frequencies of FDC.6 and FDC.7.

Correlation of Ultrastructural Data with the Immunohistopathology and Clinical Stage

Enlargement of germinal centers and deterioration of mantle zones (Table 2) appeared to be more prominent in HIV lymph nodes than in FH cases. By immunohistology, p15 and p24 HIV-1 core proteins were present in almost all of the germinal centers of most lymph nodes after HIV-1 infection. Two cases belonging to the HIV-Clu-2 cluster were negative for these proteins.

HIV-1-infected patients of clinical stage III (follicular hyperplasia) and patients of stage IVa (constitutional disease), according to CDC criteria (25), occurred in the same frequencies in both the HIV-Clu-1 and HIV-Clu-2 clusters (Table 2).

DISCUSSION

This study on germinal centers differentiates lymph nodes after HIV-1 infection into two main groups (HIV-Clu-1 and HIV-Clu-2). One group (HIV-Clu-1) clusters together with all of the cases of non-HIV-1 related follicular hyperplasia, but the other group (HIV-Clu-2) showed distinct features. The main differences are the higher incidence of blast cells and the lower incidence of centrocytes in the cases of HIV-Clu-2. These B-cell subsets are considered to represent different stages in B-cell maturation. The blast subtypes, with the small

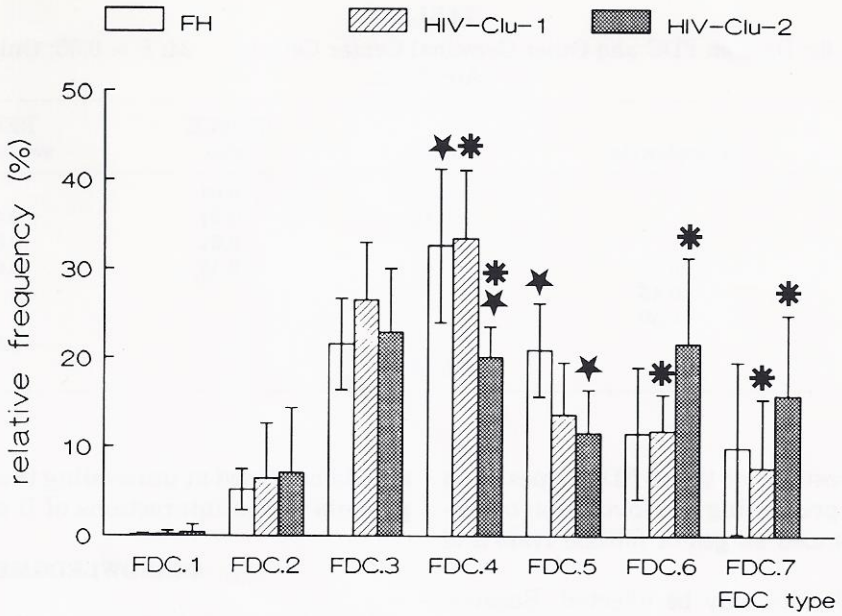

FIG. 7. Frequency distribution of the various FDC types in germinal centers of HIV-1-infected and non-HIV-1-infected patients. Corresponding symbols (★ or *) on top of the columns representing one particular FDC type indicate significantly different pairs.

centroblast as the main component, represent rather immature B cells (23) capable of proliferation; the centrocytes, which transform from centroblasts (29), can be considered as the main maturating cell type and therefore are representative of B-cell differentiation in the germinal center.

The germinal centers of lymph nodes after HIV-1 infection contain all essential cellular elements (22, 23) necessary for adequate B-cell differentiation. This allowed us to compare these data with those of control FH and normal tonsil. In the tonsil, a dark zone and a light zone can be distinguished. These zones may represent different compartments in the germinal center (23). Such zones were not evident in the present material. But, aspects of these zones were reflected in the germinal centers after HIV-1 infection. The HIV-Clu-1 cluster (composed of HIV-1 positive and HIV-1 negative FH cases) resembled that of the light zone, whereas the HIV-Clu-2 cluster was more like the dark zone. However, the predominance of blasts is not accompanied by a higher mitotic activity in these lymph nodes. These features of the HIV-Clu-2 germinal centers may reflect an altered conversion of centroblasts into centrocytes, suggesting a block in B-cell differentiation.

The relative frequency of FDC proved to be not discriminatory in the differentiation between Clu-1 and Clu-2 clusters. Subtypes of FDC were not included in the clustering procedure. Therefore it is allowed to correlate the distinct groups to their subset composition of FDC types. This analysis (Fig. 7) showed a difference between both Clu-1 and FH cases on the one hand and Clu-2 specimens on the other hand: HIV-Clu-2 cases showed a predominance of FDC.3, FDC.6, and FDC.7 and lowered proportions of FDC.4 (to a less extent FDC.5). In this aspect germinal centers in lymph nodes of the Clu-2 cluster differ from the situation in the dark zone of the tonsillar germinal center (23). In the latter low numbers FDC occur predominantly composed of the undifferentiated types FDC.1, FDC.2, and FDC.3. As judged by the condition of cell organelles (Table 3), FDC.4 and FDC.5 represent FDC with full biologic activity, whereas FDC.6 and FDC.7 presumably represent differentiated FDC showing signs of regression. So, despite the observation of a quite normal developed framework of FDC in germinal centers in the HIV-Clu-2 group, the ultrastructural features of FDC themselves suggest that the germinal center microenvironment made by FDC is altered.

Viral components may have a role in these changes of the microenvironment. As in other studies (16, 31, 32), we observed virus-like particles. Like viral antigens, these particles are probably present in the form of antigen–antibody complexes on the surface of FDC (33–37). HIV-1 proteins, especially the gp-120 envelope protein, have lytic characteristics and can be harmful to the local germinal center microenvironment (38). Because FDC express CD4 (39), they are able to interact with the gp-120 protein. In this study we observed no ultrastructural indications (a decrease in FDC number or syncytium formation) suggestive for such lytic or harmful effects on FDC. FDC can be infected by the virus (17, 31). As of yet it is unknown whether the stage of HIV-1 infection (latent or lytic, associated with virus production) affects the morphology of FDC and induces FDC subtypes of terminal differentiation (types 6 and 7). Otherwise the predominance of immature (FDC.3) and regressive FDC types (FDC.6 and FDC.7) may be related to undifferentiation of FDC into primitive reticular cells from which they

TABLE 3

Analysis of Covariance for Data on FDC and Other Germinal Center Cells ($n = 24$; $P \leq 0.05$; Only Significant r Values Are Given

	Lymphocyte	Mitosis	Blast/CC ratio	RER swelling	Lysosome incidence
FDC.4		−0.60	−0.64		
FDC.5		−0.45	−0.51	−0.41	
FDC.6			0.61	0.61	0.58
FDC.7	−0.40		0.45	0.60	0.68
Relative volume FDC	0.45				
Number of FDC/10^4 μm^2	0.50				
Nucleus/cytoplasm ratio				−0.60	−0.54
RER swelling		0.40			0.72

originate (40). The presence of these FDC types then reflects early changes preceeding the involution of germinal centers in advanced stages of follicle lysis and degeneration.

Apart from FDC, B cells may be affected. Because FDC present antigens to B cells (41–43), B cells may also serve as candidates for harmful effects of HIV-1 components or intact virus. The HIV-1 virus can act as a B-cell activator (43) and may as such induce an abnormal germinal center reaction. Our observations on B cells are not consistent with increased cell death, increased proliferation (mitotic activity), or differentiation and do not support this assumption as a cause for such an abarrant germinal center reaction. Moreover, our data indicate that the changes observed in the lymphoid germinal cell population are closely related to those occurring in FDC. Therefore, FDC appear directly involved in the genesis of an abnormal germinal center reaction after HIV infection. Because B-cell processing in the germinal center may occur in close FDC-mediated support (21, 42), it is hypothesized that the FDC framework is unable to support B-cell differentiation after HIV-1 infection (centrocyte formation from centroblasts).

In conclusion we documented that lymph nodes in the follicle hyperplasia stage after HIV-1 infection show peculiar characteristics in about two-thirds of the cases. These include a shift in the blast/centrocyte ratio toward higher proportions of centroblasts, without an elevated mitotic activity. These changes correlate with a higher incidence of FDC subtypes showing an ultrastructure indicative for poor differentiation and inactivation. Such a cell composition of germinal centers is different from that found in the physiological immune response, e.g., in germinal centers of reactive lymph nodes and tonsils. These features point to an abnormal condition of interaction between germinal centre B cells and the environment made by FDC. Apparently, factors facilitating and maintaining the differentiation of FDC are lost. It is tempting to suggest a role of HIV-1 components in the generation of these alterations. Techniques to isolate human FDC and study them *in vitro* are now available (39). Such studies should be aimed at unraveling the effects of HIV-1 components in the interactions of B cells and FDC.

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