HIV-Associated Multinucleated Giant Cells in Lymphoid Tissue of the Waldeyer's Ring: A Detailed Study

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Hyperplastic lymphoid tissues of the Waldeyer's ring in human immunodeficiency virus (HIV)–infected patients may occasionally contain multinucleated giant cells (MGCs). These cells, which are unrelated to any opportunistic infection, previously have been demonstrated to harbor significant amounts of HIV. Studies undertaken to characterize these MGCs have generated conflicting results: some reports suggested a macrophage origin, whereas others supported a dendritic cell lineage. This study was performed to determine the occurrence of MGCs in a series of adenoid/tonsil specimens from HIV-seropositive patients showing no histological evidence of opportunistic infection in order to further characterize the phenotype of these cells and to investigate the role of a viral infection in their pathogenesis. Adenoid/tonsil tissue specimens from 21 HIV-seropositive patients with no documented opportunistic infection were scrutinized for the presence of MGCs and evaluated immunohistochemically on paraffin sections by antibodies directed against various macrophage and DC antigens. These antigens included CD68, the macrophage marker 3A5, major histocompatibility complex Class II, S-100 protein, CD1a, and CD83. Additional immunostainings directed at CD21 and CD35 as well as at the HIV-associated p24 antigen were also performed. Finally, the presence of Epstein-Barr virus and human herpesvirus 8 viral sequences was investigated by *in situ* hybridization and by polymerase chain reaction analysis, respectively. MGCs were found in 14 patients (66.7%), regardless of gender, age, method of viral transmission, CD4 cell count, viral load, or ethnic group. These cells were mostly localized at the lymphoepithelium layer of the tonsillar crypts and, to a lesser extent, in the interfollicular areas of the underlying lymphoid tissue, which consistently exhibited features of follicular hyperplasia. Phenotypically, MGCs were found to be CD68$^+$, 3A5$^+$, major histocompatibility complex Class II$^+$, S-100 protein$^{+/-}$, CD1a$^-$, CD21$^-$, CD35$^-$, and CD83$^-$. Although the HIV-associated p24 protein was consistently present in the cytoplasm of these cells, no sign of Epstein-Barr virus or human herpesvirus 8 infection could be demonstrated. Consequently, our study didn't show any conclusive evidence to support that MGCs in hyperplastic lymphoid tissues of the Waldeyer's ring from HIV-seropositive patients originated from dendritic cells. The definite nature of these cells has yet to be elucidated, but it is plausible that they simply represent activated macrophages that are infected with HIV present in the oropharyngeal secretions during the circulation of their precursor through the lymphoepithelium area of adenoids and tonsils.

KEY WORDS: Adenoid, EBV, HHV-8, HIV infection, Multinucleated giant cell, Tonsil.

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As in the lymph nodes, the lymphoid tissue of the Waldeyer's ring (WR) may be infected by the human immunodeficiency virus (HIV; 1, 2). In this setting, miscellaneous pathological changes that are closely related to this retroviral infection or to its various complications have been reported (1, 2). Among these, a somewhat underrecognized and intriguing feature is the occasional presence of multinucleated giant cells (MGCs) in hyperplastic lymphoid tissue of the WR (2–10). These cells mostly occur isolatedly or in small clusters at the lymphoepithelium layer of the tonsillar crypts and strikingly resemble those previously described in brain and spinal cord tissues of patients with acquired immune deficiency syndrome (AIDS)–related encephalopathy and myelopathy, respectively

(2–10). Similar cells were also documented in a case of nodal lymphoma associated with AIDS (11), in HIV-associated lymphoepithelial cysts of the parotid gland (12), and more recently, in the colonic mucosa of an HIV-seropositive man (13). These MGCs seem to be unrelated to any opportunistic agent, Epstein-Barr virus included, and they are known to harbor significant amounts of both HIV genomic sequences and viral products (3–13). These cells are morphologically distinct from Warthin-Finkeldey polykaryocytes and rather look more like Langhans'-type MGCs (9). Studies undertaken in order to assess their lineage have generated conflicting results: some reports advocated a macrophage origin (8, 10, 13), whereas others claimed a dendritic cell (DC) lineage, not only because of their S-100 protein and p55 (fascin) expression but also because of their striking resemblance to the virus-producing DC–T cell syncytia that are observed in *in vitro* studies (4–7, 14, 15). In an attempt to resolve the controversy surrounding the nature of these cells, we performed a phenotypic study using a panel of antibodies directed against various macrophage, follicular dendritic cell (FDC), and DC antigens. In addition, the issue of a viral infection in the pathogenesis of these MGCs was also addressed by searching for the presence of HIV, Epstein-Barr virus (EBV), and human herpesvirus 8 (HHV-8) viral products.

MATERIALS AND METHODS

Twenty-four adenoid/tonsil tissue samples from 21 patients known to be seropositive for HIV virus and showing no histological evidence of opportunistic infection were collected for this study. Tissue samples were obtained either from biopsies or from surgically resected specimens. All were fixed in formalin, conventionally processed, and embedded in paraffin wax. Histological sections were stained with hematoxylin and eosin. Each slide was carefully reviewed and scrutinized for the presence of MGCs. The main clinical data and the most relevant morphological features of the lymphoid tissue were recorded for each case (Tables 1 and 2).

Immunohistochemistry

Immunophenotypic studies were performed on paraffin sections using a Ventana Nexes Staining System (Ventana Medical Systems, Tucson, AZ). Antibodies directed against the following antigens were used: HIV-related p24 protein, CD21, CD35, S-100 protein, CD68 (PG-M1 clone; 16), major histocompatibility complex (MHC) Class II (DAKO A/S Glostrup, Glostrup, Denmark); CD1a (Immunotech SA, Marseille, France); and CD83 (Serotec, Oxford, UK). In addition, the monoclonal antibody 3A5

(Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK) that recognizes a formalin-resistant epitope present on macrophages and monocytes but virtually absent from DCs was also used for this study (17).

Detection of EBV by *In Situ* Hybridization

To investigate the possible role of EBV infection in the pathogenesis of MGCs, an *in situ* hybridization for EBV-encoded RNA transcripts was performed, using a supersensitive *in situ* hybridization detection system (BioGenex, San Ramon, CA).

Detection of HHV-8 by Polymerase Chain Reaction Analysis

Paraffin-embedded tissues were cut into 5-μm-thick sections. Three sections of each samples were put into an Eppendorf tube and deparaffinized twice by adding 1 mL of toluol, incubated for 5 minutes, and then centrifuged for 5 minutes at 13,000 rpm. Samples were then treated twice by adding 1 mL ethanol 100%, incubated for 5 minutes, and centrifuged for 5 minutes at 13,000 rpm.

The pellet was then resuspended in TE buffer (20 mM Tris, 1 mM EDTA; pH 8.5) containing 200 μg/mL proteinase K (Roche Molecular Biochemicals, Brussels, Belgium) and incubated overnight at 55° C. It was then heated at 95° C for 10 minutes to inhibit proteinase K. After a last centrifugation at 13,000 rpm for 5 minutes, 5 μL of the supernatant was used for polymerase chain reaction (PCR) amplification.

As a control for gene amplification, the following PCO3 and PCO4 primers derived from the β-globin gene, giving an amplified fragment of 110 base pairs (bp), were used: PCO3: 5′-ACACAACTGTGTTCACT-AGC-3′ and PCO4: 5′-CAACTTCATCCACGTTCAC-C-3′.

The PCR mixture contained 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl$_2$, 200 μM dNTP, 10 picomoles of each primer, and 1 U DNA *Taq* polymerase (Promega, A.J. Leiden, The Netherlands) in a final volume of 50 μL. Reactions were incubated in a Perkin-Elmer Thermocycler 2400 for 35 cycles (denaturation: 1 min at 94° C, annealing: 1 min at 55° C, elongation: 1 min at 72° C). After amplification, 18 μL of the PCR product was run on a 2% agarose gel stained with ethidium bromide.

Samples showing amplifiable DNA were then investigated for their putative HHV-8 content. Primers derived from the HHV-8 genome promoting the amplification of a 111-bp product from 987 to 1097 bp of the published sequence (18) were used as follows: forward primer, 5′ AGCCGAAAGGATTCCACCAT-3′ and reverse primer, 5′-GTACACCAACAGCTGCTGCA-

TABLE 1. Summary of Clinical Features

Case Number	Sex (M/F)	Age (Years)	Anatomic Location	Clinical Data	Ethnic Group/Mode of HIV Transmission	CD4 Count (μl)/viral load (copies/mL)*	HIV Status at Time of Biopsy**/Follow-Up
1	M	38	Palatine tonsils	Enlargement	Caucasian/bisexual	110/NA	C/DOD within the year
2	M	30	Adenoids	Mass, recurrent otitis	Caucasian/bisexual	NA/NA	NA/lost for follow-up
3	M	36	Palatine tonsils	Enlargement	Caucasian/NA	NA/NA	NA/NA
4	M	27	Adenoids	Enlargement	Caucasian/homosexual	NA/NA	A/AIDS 8 years later
5	M	49	Palatine tonsils	Enlargement	Caucasian/homosexual	389/NA	B/alive
6	F	48	Adenoids	Enlargement	African/heterosexual	226/NA	B/alive 2 years later, then lost for follow-up
7	F	21	Adenoids, palatine tonsils	NA	African/heterosexual	<100/NA	B/alive
8	M	29	Adenoids	Enlargement	Caucasian/homosexual	NA/NA	A/DOD 3 years later
9	F	32	Adenoids	Enlargement, postnasal discharge	Caucasian/heterosexual	945/NA	A/alive
10	F	38	Adenoids, palatine tonsils	NA	African/heterosexual	135/NA	A/alive 1 year later
11	M	27	Palatine tonsils	NA	Caucasian/homosexual	62/NA	A/alive 1 year later
12	F	33	Adenoids	Enlargement	Caucasian/heterosexual	177/<4000	B/DOD 3 years later
13	M	28	Adenoids	Polypoid mass, otitis serosa	Caucasian/bisexual	229/70000	A/alive 1 year later
14	M	35	Adenoids, palatine tonsils	Enlargement, recurrent tonsillitis	African/heterosexual	426/10670	A/alive 1 year later
15	M	43	Adenoids, palatine tonsils	Enlargement	African/heterosexual	17/96459	C/alive
16	F	4	Adenoids	Enlargement, otitis serosa	African/feto-maternal	1280/<400	A/alive
17	F	4	Adenoids, palatine tonsils	Nasopharyngeal obstruction	African/feto-maternal	828/1298	A/B 1 year later
18	M	11	Adenoids, palatine tonsils	Enlargement	African/feto-maternal	343/119000	A/alive
19	M	6	Adenoids, palatine tonsils	Enlargement	African/feto-maternal	590/104000	A/alive
20	F	25	Adenoids	Slow growing mass	African/heterosexual	159/49500	A/alive
21	F	30	Adenoids	Enlargement	African/heterosexual	194/NA	A/alive

NA, not available; DOD, dead of disease.

* The viral load was determined by nucleic acid sequence based analysis (NASBA).

** The HIV status at time of biopsy was defined according to the 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults (Centers for Disease Control and Prevention (CDC), MMWR 1992; 41(No. RR-17).

3′. The PCR mixture contained 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.1% Triton X-100, 200 μM dNTP, 12.5 picamoles of each primer, and 1 *Taq* Bead Hot Start polymerase wax bead containing 1.25 U *Taq* polymerase (Promega) in a final volume of 25 μL. PCR conditions were as follows: 5 minutes at 94° C; 40 cycles of 1 minute at 94° C, 1 minute at 55° C, and 1 minute at 72° C; a final elongation of 7 minutes followed at 72° C. After amplification, 18 μL of PCR product was run on a 2% agarose gel stained with ethidium bromide.

Positive control for HHV-8 presence consisted of paraffin-embedded tissue sections that originated from an HIV-seropositive patient with a known HHV-8–positive Kaposi's sarcoma and that was processed similarly to the tonsil samples.

RESULTS

In most cases, the biopsy or the surgical resection was performed because of a clinically unexplained enlargement of the adenoids and/or tonsils (Table 1).

MGCs were found in 14 patients (66.7%), seven men and seven women. The age of these patients ranged from 4 to 43 years (mean age, 27.6 y; median age, 30 y). In each case, the number of MGCs varied from 1 to more than 10 per slide. In all of these cases, the histological appearance of the adenoid/tonsillar lymphoid tissue was that of a follicular hyperplasia with prominent germinal centers, occasional irregular outlines, and/or folliculolysis.

TABLE 2. Summary of Pathologic Findings

Case Number	Histological Features	3A5	HLA-DR	S-100 Protein	CD68	CD1a	CD21	CD35	CD83	p24	EBV	HHV8
1	Atrophy											
2	FH, MGC	+	+	+	+	−	−	−	−	+	−	NA
3	FH											
4	FH, MGC	+	+	−	+	−	NA	NA	−	NA	NA	NA
5	FH											
6	FH											
7	FH, MGC	+	+	−	+	−	−	−	−	+	−	−
8	FH, MGC	+	NA	+	+	−	−	−	−	+	−	NA
9	FH, MGC	+	−	−	+	−	NA	NA	NA	NA	NA	−
10	FH, MGC	+	+	+	+	−	−	−	−	+	−	−
11	FH											
12	FH, MGC	+	+	−	+	NA	−	−	−	+	−	NA
13	FH, MGC	+	+	−	+	−	−	−	−	+	NA	−
14	FH, MGC	+	NA	NA	+	−	NA	NA	NA	NA	NA	NA
15	FH, MGC, DLBCL	+	+	+	+	−	−	NA	−	NA	NA	−
16	FH											
17	FH, MGC	+	+	−	+	−	−	−	−	−	−	NA
18	FH, MGC	+	+	+	+	−	−	NA	−	+	NA	NA
19	FH											
20	FH, MGC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	FH, MGC	+	+	NA	+	NA	−	−	−	−	−	NA

FH, follicular hyperplasia; MGC, presence of multinucleated giant cells; DLBCL, diffuse large B-cell lymphoma; NA, not available.

MGCs were mostly localized in or just beneath the lymphoepithelium (Fig. 1, A-B). In rare instances, they were also encountered in the interfollicular areas or in close contact with the mantle zone of the follicles. Simultaneous involvement of both adenoids and/or tonsils by MGCs was seen in three patients, whereas in three patients, MGCs were found in successive samples in a few months' interval. In one case, these cells were observed in the residual and hyperplastic tonsillar tissue surrounding a diffuse large B-cell lymphoma.

Immunohistochemical analysis of the MGCs showed constant expression of CD68 (13/13), 3A5 (13/13; Fig. 2), and MHC Class II antigens (10/11). S-100 protein expression was seen in 5/11 cases (45%; Fig. 3). In these cases, the intensity of the immunostaining was occasionally weaker than that of DCs found in both lymphoepithelium and interfollicular areas. No CD1a, CD21, CD35, or CD83 immunoreactivity could be demonstrated (respectively, 0/11, 0/10, 0/8, and 0/10 analyzable cases; Fig. 4). In all the cases tested, the staining with monoclonal antibody directed against the HIV p24 protein characteristically outlined the FDC network in the germinal centers as well as some lymphoid cells in the interfollicular areas. Its immunoexpression by MGCs was recorded in 7/9 cases (77%). No EBV-encoded RNA transcript could be demonstrated in the MGCs (0/7), whereas scattered EBV-encoded RNA positive lymphocytes were frequently found in the surrounding lymphoid tissue. No HHV-8 DNA could be demonstrated by PCR analysis in the five cases suitable for such investigation.

DISCUSSION

This work further defines the nature of MGCs that are found in hyperplastic lymphatic tissue of the WR in HIV-seropositive patients. These particular cells have been studied only recently, and studies dealing with that matter are scarce (3–6, 8–10) and often originate from the same investigation group (4–6). Because of their S-100 protein and fascin expression on one hand and their striking resemblance to HIV-producing DC-T cell syncytia that are observed in *in vitro* studies on the other hand, some authors have suggested that MGCs originated from DCs (4–7, 14, 15). The large number of cases collected in our study, together with the use of a broad panel of antibodies that specifically recognize distinct markers of DCs, FDCs, and macrophages, allow additional comments to be made.

MGCs were found in 66.7% of our patients, irrespective of gender, age, method of viral transmission, CD4 cell count, viral load, or ethnic group. This percentage, which is in agreement with those of published series, can give a reasonable idea of the overall incidence of this feature in HIV-associated lymphatic hyperplasia of the WR. In this respect, it is worth noting that most of our negative cases were, in fact, small biopsies with relatively small amounts of analyzable tissue.

In our hands, MGCs were found to be CD68^{+}, 3A5^{+}, MHC Class II^{+}, S-100 protein^{+/−}, CD1a^{−}, CD21^{−}, CD35^{−}, and CD83^{−}. With regard to the specificity of these various markers, both PG-M1 and 3A5 antibodies recognize fixative-resistant epitopes that are normally expressed by macrophages (16, 17). These monoclonal antibodies do
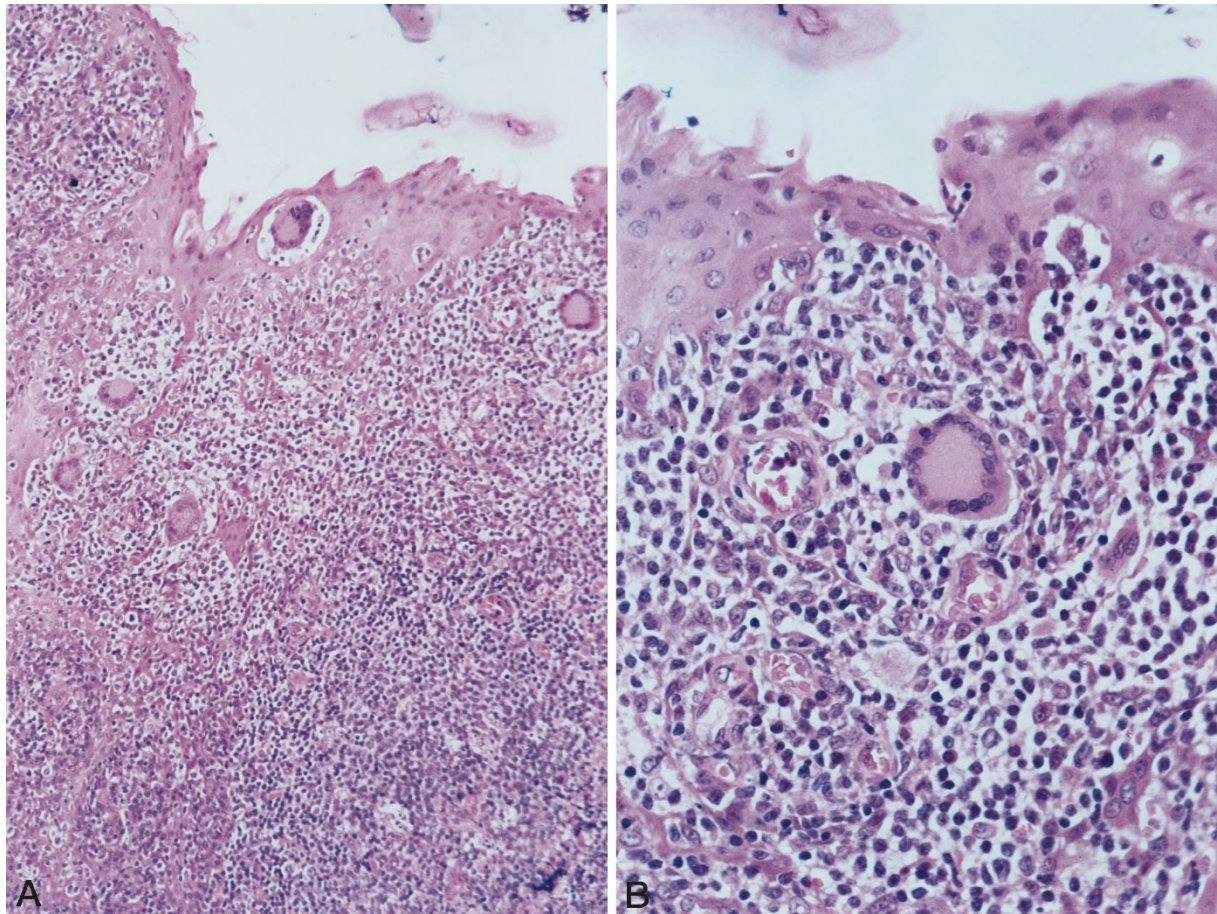
FIGURE 1. Hematoxylin- and eosin-stained sections (**A**, **B**) of a tonsillectomy specimen featuring numerous multinucleated giant cells. These characteristic cells are localized in proximity to the lymphoepithelium area.
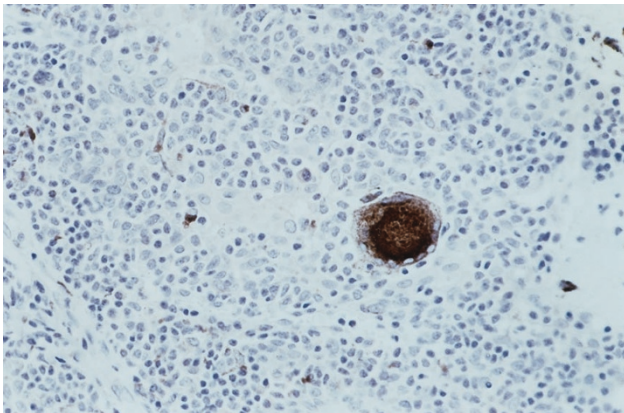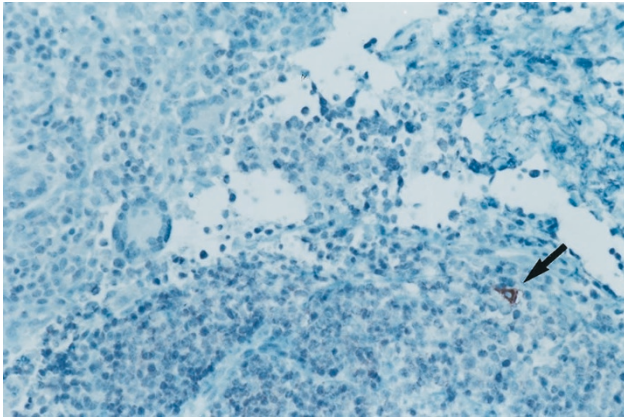

FIGURE 2. Immunostaining with 3A5 antibody. This multinucleated giant cell shows a strong immunoreactivity.
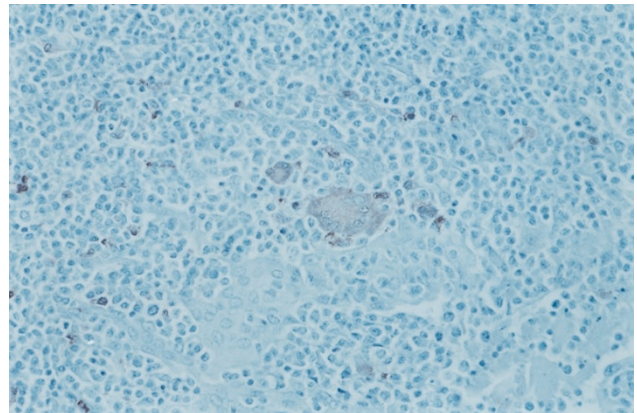
FIGURE 3. Immunostaining with antibody directed against S-100 protein. In this case, multinucleated giant cells were found to be weakly immunoreactive. Note that surrounding dendritic cells also yield staining.

not react with antigen-presenting cells such as interdigitating reticulum cells, Langerhans cells (LCs), or FDCs (16, 17). In contrast, CD83 is a DC lineage antigen (19–21). It belongs to the immunoglobulin superfamily, and its expression on DCs is upregulated after activation (19, 20). This molecule is also expressed on other cell types such as LCs, FDCs, germinal centers, and activated blood lym-

phocytes (19). CD1a is a useful marker of DCs that belong to the LC family (21, 22), whereas CD21 and CD35 preferentially stain FDCs (22). MHC Class II antigens are expressed on all B cells, activated T lymphocytes, monocytes, and macrophages and on all DC populations, including interdigitating reticulum cells, LCs, and FDCs (20, 21). As a result, we

FIGURE 4. Immunostaining with antibody directed against CD83. This multinucleated giant cell is characteristically negative. Note a strongly stained dendritic cell (arrow).

think that the inconstant or weak S-100 protein immunoreactivity that characterizes MGCs and their lack of CD83 expression, together with their PG-M1 and 3A5 reactivity, are in favor of a macrophage phenotype.

Notwithstanding that S-100 protein expression is usually considered a consistent feature of DCs (4–6), this marker lacks specificity because it can also be expressed by various histiocyte subsets (23, 24). Furthermore, its expression by macrophages has been documented in various diseases such as Rosai-Dorfman disease (25), multibacillary leprosy (26), and HIV infection (27, 28). In all of these conditions, this apparently aberrant expression was demonstrated to be associated with down- or up-regulation of other markers, thereby suggesting functional alterations that are probably related to the underlying immune or infectious disorder (25–28).

Because of the consistent presence of HIV in MGCs in our study as well as in those of others (3–6, 8–13) and because no EBV or HHV-8 could be demonstrated in these cells, we think that the morphology as well as the phenotype of MGCs may be a direct consequence of their infection by this retrovirus. In this respect, the presence in our study of MGCs that are simultaneously p24 negative or weakly positive and S-100 protein negative (Cases 7, 12, and 17) is interesting. This observation, which suggests that a significant viral load in the macrophage is necessary to induce phenotypic changes such as S-100 protein expression, is in keeping with the knowledge that HIV-infected macrophages display significant alterations of their immune functions, despite their relative resistance to the virus cytopathic effects (29, 30). Moreover, HIV-1 infection of primary monocytes but not of monocyte-derived macrophages has been demonstrated to induce cellular alterations as well as generation of MGCs in *in vitro* studies (31).

The preferential localization of MGCs in the lymphoepithelium and, to a lesser extent, in the interfollicular zones, in conjunction with a follicular hyperplasia suggests a specific role for these cells in the tonsillar HIV-associated immune response. Both lymphoepithelium and interfollicular areas are indeed specialized trafficking sites at which antigens can be processed and/or presented by antigen-presenting cells to other immune effector cells to initiate a specific response (14, 32). In this regard, it is worth noting that macrophages are normally present in the lymphoepithelium area, where they maintain close relationships with M cells (33). The latter are a highly specialized cellular component of the mucosa-associated lymphoid tissue that acts as a gateway for various antigens that are present in the body cavities, like the nasopharynx (33). Such antigens are processed and/or forwarded through the cytoplasmic invaginations of M-cells, which subsequently present them to the various immune effector cells circulating in the lowest part of the lymphoepithelium (14, 32–34). In keeping with the findings of Fais *et al.* (31), we can therefore speculate that MGCs probably result from the transmigration and homotypic fusion of blood monocytes that are infected by HIV present in the nasopharyngeal secretions during the circulation of these cells in the lymphoepithelium area.

The persistence of MGCs in the same anatomic sites, at several months' interval, also suggests that this phenomenon is chronic. This finding is consistent with the knowledge that macrophages are a long-lived HIV reservoir, thereby providing a continuous source of virus for *de novo* infection of susceptible target cells that reside and/or circulate in the lymphoid tissue (35–37).

Although the DC origin of MGCs is an attractive supposition, given the lack of specificity of both S-100 protein and fascin, because they are also expressed by macrophages or other cell types (23–28, 38), we think that assessing the DC lineage by merely studying these markers is insufficient to validate such a hypothesis. Whatever their precise ontogeny, the detection of these very characteristic cells in hyperplastic lymphatic tissues of the WR at least has the merit of alerting the pathologist to the possibility of an underlying HIV infection.

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