Primary Human Cytomegalovirus Infection Induces the Expansion of Virus-Specific Activated and Atypical Memory B Cells

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Background. Although neutralizing antibodies play a central role in the control of cytomegalovirus (CMV) dissemination, little is known about the response of B lymphocytes to primary human CMV infection.

Methods. The proportion, phenotype, specificity, and functionality of B-cell subsets were studied in a cohort of pregnant women with primary CMV infection. CMV-seronegative pregnant women, as well as CMV-seronegative and CMV-seropositive healthy adults, were included as controls.

Results. Primary CMV infection was associated with a sustained expansion of activated (CD27+CD20+CD21low) and atypical (CD27−CD20+CD21low) memory B cells (MBCs). Both subsets expressed an effector phenotype, and their proportions were correlated with viremia. Activated MBCs expressed high levels of activation markers and included high frequencies of tumor necrosis α (TNF-α)–producing cells, whereas atypical MBCs expressed high levels of inhibitory receptors and had low TNF-α responses. Fluorescent-labeled antigen experiments indicated that activated and atypical MBCs were enriched in CMV-specific cells.

Conclusions. Primary CMV infection mobilizes a large pool of memory B cells that includes activated and atypical MBCs. The functional regulation of CMV-specific MBCs may limit the production of antibodies and the control of viral dissemination.

Keywords. cytomegalovirus; primary infection; atypical memory B cells; activated memory B cells.

Human cytomegalovirus (CMV), a member of the BetaHVirus subfamily, establishes lifelong persistence following primary infection. CMV infection is usually asymptomatic in immunocompetent adults but causes severe morbidity and mortality in patients with acquired immunodeficiency. In addition, CMV is the most important cause of congenital infection and causes severe sequelae, including deafness and mental retardation [1, 2].

Most of the research on immunity to CMV has been focused on T lymphocytes, and very little is known about the response of B lymphocytes to CMV infection [3, 4]. However, clinical and animal studies indicate that antibodies play an important role in protection. In the mouse, neutralizing antibodies protect against primary CMV infection and reactivation by limiting viral dissemination and viral spread within tissues [5–8]. In addition, transfer of B cells from immune animals reduces viral load and protects against lethal infection [9]. In clinical studies, high titers of anti-CMV neutralizing antibodies correlated with a favorable outcome in several patient populations [10–14]. More direct evidence comes from vaccine studies showing protection against infection and viral replication following immunization with an adjuvanted CMV glycoprotein B vaccine inducing primarily neutralizing antibodies [15, 16].

Following primary infection, CMV disseminates to most organs and is excreted for several months in
body fluids. Studies of persistent infections have shown that prolonged exposure to high antigen loads can have a profound impact on B-cell populations [17–20]. In HIV-infected patients, viral replication is associated with the expansion of several B-cell subsets, including activated memory B cells (MBCs) and atypical MBCs. Activated MBCs express reduced levels of the complement receptor CD21. Although distinct from plasma cells, activated MBCs have a high antibody secretion capacity [21, 22]. Like activated MBCs, atypical MBCs express low levels of CD21 but lack the expression of the classical memory marker CD27. Atypical MBCs express a large range of inhibitory receptors that reduce their effector functions and proliferative potential [23, 24]. These features are analogous to those of exhausted T lymphocytes induced by persistent viral infections and are considered to play an important role in the defective B-cell responses associated with human immunodeficiency virus (HIV) infection [19, 25].

This study was undertaken to evaluate the impact of CMV infection on B-cell subsets. In a cohort of pregnant women who received a diagnosis of primary CMV infection, we observed large and sustained expansions of both activated and atypical MBCs that are enriched in virus-specific cells.

MATERIALS AND METHODS

Study Population
This study was approved by the Ethics Committee of the Erasme Hospital, Brussels. Pregnant women referred with a diagnosis of primary CMV infection were recruited following receipt of their written informed consent. Diagnosis of primary infection was based on documented immunoglobulin G (IgG) seroconversion or, in case of unknown status at the beginning of the pregnancy, increasing titers of CMV-specific IgG in sequential serum samples in the presence of detectable immunoglobulin M (IgM) or the association of clinical symptoms and biological alterations in the presence of IgM only [26]. CMV viremia was assessed using a qualitative in-house diagnostic polymerase chain reaction (PCR) assay targeting the pp150 CMV gene. PCR was performed on a Light Cycler480 PCR system (Roche Diagnostics) with the forward primer 5′-CTG ATG AGG TTT GGG CTT TAA-3′, the reverse primer 5′-TCC GAG GAG TCG TCG TCT T-3′, and the probe 5′-FAM_CAA ACT GCA GAG TCA CCG GTC GAA-TAMRA-3′. Blood samples were collected from 47 women at the time of recruitment and from 12 women at the time of delivery. In addition, blood samples were collected from 23 CMV-seronegative pregnant women, as well as from 21 CMV-seropositive and 12 CMV-seronegative healthy donors, by ImmuneHealth (CHU Tivoli, La Louvière, Belgium) following receipt of their written informed consent. CMV serology was determined using commercially available enzyme-linked immunosorbent assay kits (VIDAS CMV IgG, Biomerieux, or ETI-Cytok-G PLUS, Diasorin).

Phenotyping of B-Cell Subsets
PBMC were purified from peripheral blood by density gradient centrifugation (Lymphoprep, Nycomed Pharma) and cryopreserved until use. After thawing, cells were stained with monoclonal antibodies, fixed using Cellfix (BD Biosciences) and acquired with a Cyan ADP L9x cytometer (DakoCytomation). The following antibodies were used following titration: CD3 V500, CD19 PeCy7, CD27 V450, CD11c PE, CD95 PE, Ki-67 PE, CD62L PE, LAIR1 PE, and IgG PE (all from BD Biosciences); CD20 PerCP, CD21 FITC, CD10 APC, CD80 PE, CD22 PE, CXCRI4 PE, and CD85J PE (all from BioLegend); CCR7 PE and CXCRI5 PE (both from R&D systems); and CD72 PE (Exbio Praha). PE-conjugated FcRL4 antibody (clone A1) [27] was kindly provided by Prof Satoshi Nagata (Sanford Research Center, Sioux Falls, SD). Intracellular Ki-67 staining was performed following incubation with Cytotox/Cytoperm (BD Biosciences). B-cell subsets were identified using the gating strategy described in Figure 1A. B cells were defined as CD3− and CD19+ live lymphocytes. Within the CD27+ B-cell population, CD20 and CD21 expression was used to discriminate classical MBCs (ie, CD27+CD20+CD21+ B cells), activated MBCs (ie, CD27+CD20+CD21low B cells), and plasma cells (ie, CD27−CD20+CD21− B cells). Within CD27− B cells, CD10 expression was used to identify immature B cells. Among CD27−CD10− B cells, CD20 and CD21 expression was used to discriminate naive B cells (CD27−CD20−CD21−) and atypical MBCs (CD27−CD20+CD21low). A fluorescence-minus-one strategy was used to discriminate between positive and negative cells within the different subsets [28].

Cytokine Production by B-Cell Subsets
Tumor necrosis factor α (TNF-α) production by B-cell subsets was measured following in vitro stimulation with CpG ODN 2006 (2.5 µg/mL, InvivoGen), CD40L (1 µg/mL, Cell Signaling Technology), and goat anti human IgG/IgM/IgA (5 µg/mL, Jackson ImmunoResearch). GolgiPlug (BD Biosciences) was added after 2 hours of stimulation. After 18 hours of stimulation, cells were stained with the viability marker Live/Dead ECD (Life Technologies) and with the following titrated antibodies: CD3 V500, CD19 AF700, CD27 V450 (all from BD Bioscience), CD20 PerCp and CD21 FITC (both from Biolegend). Cells were then permeabilized with Cytotox/Cytoperm (BD Biosciences), stained with TNF-α PE antibody (BD Biosciences), fixed with Cellfix (BD Biosciences), and acquired using an LSR II flow cytometer (BD Biosciences).

Analyses of Antigen-Specific B Cells
To identify antigen-specific B cells, a dual staining protocol with fluorescent antigens was used [29]. Two polypeptides containing immunodominant domains of CMV tegument proteins (pp150/2-pp52/3 and pp150/7-pp150/1) [30, 31] and tetanus toxoid (a kind gift of GlaxoSmithKline Vaccines, Rixensart,
Figure 1. Expansion of activated and atypical memory B cells during primary cytomegalovirus (CMV) infection. B-cell subsets were identified using the markers and the gating strategy depicted in panel A. B, The proportions of B-cell subsets were measured in 12 CMV-seronegative adults, 38 pregnant women with primary CMV infection, and 16 adults with chronic infection. Results are expressed as the percentage of total CD19+ B cells in individual subjects. Bars indicate median frequencies. **P < .001; ***P < .0001. Abbreviations: BC, B cell; MBC, memory B cell.
Belgium) were biotinylated using the EZ-Link Micro NHS PE04-Biotinylation kit (Thermo Fisher Scientific). Antigen biotinylation was measured using the Fluorescence Biotin Quantitation kit (Thermo Fisher Scientific). Following biotinylation, antigens were incubated with PE- or V450-conjugated streptavidin (BD Biosciences) at a molar ratio of 4 biotins per 1 streptavidin. Before labeling, B cells were isolated by negative selection, using the StemCell Easy Sep kit (STEMCELL Technologies). Cells were then stained with the following titrated antibodies: CD19 ECD (Beckman Coulter/Immunotech), CD27 FITC (BD Biosciences), immunglobulin D (IgD) V500 (BD Biosciences), CD10 APC (BioLegend), CD20 PerCp (BioLegend), and CD21 PeCy7 (BD Biosciences), along with the combination of PE- and V450-conjugated antigens. Cells were then fixed with Cellfix (BD Biosciences) and acquired using a Cyan ADP LX9 cytometer. At least 50 000 B cells were acquired, and B cells that were IgD - class switched and double positive for PE and V450 were considered antigen specific [29]. Specificity of the staining was assessed using specimens from CMV-seronegative subjects (Figure 6A) and by performing inhibition experiments with the unlabeled native antigens (data not shown).

Statistical Analyses
Analyses of flow cytometry data were performed using FlowJo software (Tree Star, Ashland, OR). Statistical analyses were performed using the GraphPad Prism software (version 5.03). Multiple comparisons between groups were made using the nonparametric Kruskal–Wallis test with the Dunn posttest, while comparisons between subsets and between time points were made using the Wilcoxon paired test. Correlations between subsets were made using the Spearman test. Differences were considered statistically significant at P values of <.05.

RESULTS

Primary CMV Infection Induces the Expansion of Activated and Atypical MBCs
The impact of CMV infection on B-cell subsets was studied in pregnant women with a diagnosis of primary infection, in healthy adults with chronic CMV infection, and in CMV-seronegative adults. The characteristics of the study population are shown in Supplementary Table 1. As shown in Figure 1B, the proportions of immature, naive, classical memory, and plasma cells were comparable in the 3 study groups. In contrast, primary infection was associated with an expansion of activated and atypical MBC subsets. Proportions of activated MBCs were about 2-fold higher in women with primary infection (median, 7.7%) compared with chronically infected (4.0%) and seronegative (3.7%) adults. Similarly, atypical MBCs represented 6.4% of total B cells in women with primary infection and 3.4% and 2.3% in chronically infected and seronegative adults, respectively. The analysis of CMV-seronegative pregnant women indicated that pregnancy itself did not have a significant impact on the proportions of MBC subsets (Supplementary Figure 1). These results indicate that primary and not chronic CMV infection induces the expansion of activated and atypical MBC. As shown in Figure 2, the proportions of activated and atypical MBCs were correlated during primary and not chronic infection and were higher in women who were viremic at the time of sampling, compared with women with no detectable viremia, indicating that the expansion of MBC subsets was related to the activity of CMV infection. The comparison of the proportions of B-cell subsets at the time of diagnosis of primary infection and at the time of delivery (median interval, 118 days) indicated that the expansions of activated and atypical MBCs were sustained for several months (Supplementary Figure 2). In contrast, the proportions of naive B cells decreased and the proportions of naive B cells increased during the follow-up, suggesting an increased transition from immature to naive B cells (Supplementary Figure 2).

Atypical and Activated MBCs Have an Effector Phenotype
Studies of HIV-infected patients have shown that atypical MBCs have an effector phenotype characterized by the downregulation of lymph-node-homing receptors and by the upregulation of chemokine receptors promoting homing to the tissues [18, 24]. The expression of a similar phenotype by activated MBC could be anticipated but has not yet been described. To characterize the populations of atypical and activated MBCs during primary CMV infection, we compared their expression of chemokine receptors with that of naive B cells and of classical MBCs. As shown in Figure 3, atypical MBCs expressed lower levels of lymph-node-homing receptors, including CCR7, CD62L, CXCR4, and CXCR5, compared with naive and classical MBCs. A similar phenotype was expressed by activated MBCs. In contrast, atypical and activated MBCs expressed high levels of tissue-homing receptors. Both cell subsets expressed higher levels of CXCR3 than classical MBCs (data not shown) and higher levels of CD11c than naive and classical memory B cells. Of note, atypical MBCs expressed significantly higher levels of CD11c than activated MBCs. In agreement with their memory phenotype, activated and atypical MBCs included higher proportions of IgG + cells than naive B cells (Figure 3). Of note, these proportions were lower in atypical than in activated MBCs. Together, these results indicate that during primary CMV infection, the expanded populations of atypical and activated MBCs express an effector phenotype.

Atypical Memory B Cells Express High Levels of Inhibitory Receptors
A central characteristic of atypical MBCs is the expression of high levels of inhibitory receptors [18, 24]. CD22, CD72, and FcRl4 are coreceptors inhibiting BCR signaling [32–34]. LAIR1 and CD85j are broadly expressed inhibitory receptors that decrease BCR-induced Ca2+ mobilization [35, 36]. As
shown in Figure 4, the expanded population of atypical MBCs expressed high levels of several inhibitory receptors during primary CMV infection. Atypical MBCs expressed higher levels of CD22, CD72, and LAIR1, compared with both classical and activated MBCs and higher levels of CD85j, compared with classical MBCs. The levels of inhibitory receptors expressed by atypical MBCs were close to those expressed by naive B cells, except for CD85j, which was expressed at lower levels by naive cells. Activated MBCs expressed the lowest levels of inhibitory receptors among the 4 B-cell subsets, except for CD85j, which was expressed at a level between those expressed by classical and atypical MBCs. The inhibitory receptor FcRL4 was expressed at higher levels by all memory subsets, compared with naive cells, but in contrast to findings for HIV-infected patients [24], the highest levels were not observed on atypical MBCs. Interestingly, higher expression of inhibitory receptors were detected on atypical MBCs during primary CMV infection, compared with chronic CMV infection (mean fluorescence intensity of CD22, 125.5 vs 64.7 [P = .0005]; of CD72, 275.5 vs 148 [P = .0014]; of LAIR-1, 86.2 vs 60.2 [P = .005]; and of CD85j, 57.6 vs 32.2 [P = .0194]; percentage of FcRL4+ cells: 4.8% vs 2.2% [P = .0032]), suggesting a more intense functional regulation associated with active CMV replication.

To evaluate the level of activation of MBC subsets during primary CMV infection, their expression of Ki-67 (a marker of cycling cells), CD95, and the costimulatory molecule CD80 was measured. As expected, activated MBCs expressed higher levels of the 3 activation markers, compared with classical MBCs (Figure 4). In contrast, the levels of activation markers were lower in atypical MBCs than in activated MBCs and were similar to the levels expressed by classical MBCs. These results are in line with those observed in HIV-infected patients and suggest that, during primary CMV infection, atypical MBCs have a limited degree of activation [24]. To evaluate the relative capacity of atypical MBC to respond to polyclonal activators, their

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**Figure 2.** Frequencies of activated and atypical memory B cells (MBCs) are correlated during primary cytomegalovirus (CMV) infection and correlate with viremia. A. The correlations between the proportions of activated and atypical MBCs were calculated in 38 women with primary CMV infection and in 16 adults with chronic CMV infection. B. Proportions of activated and atypical MBCs in pregnant women with detectable viremia (n = 14) or undetectable viremia (n = 22). *P < .05; ***P < .0001. Abbreviations: BC, B cell; MFI, mean fluorescence intensity.
production of TNF-α was measured. As shown in Figure 5A, a significant production of TNF-α was detected in activated MBC (4.46%) in the absence of in vitro stimulation whereas low proportions of TNF-α⁺ cells were detected in atypical MBC (1.1%) and in the other B-cell subsets. Following short-term polyclonal activation, the proportion of TNF-α⁺-producing cells increased in all B-cell subsets. The highest proportions of cytokine-producing cells were detected in classical and activated MBCs (20.3% and 15.82%, respectively), whereas low and similar proportions were detected in atypical memory and naive B cells (5.82% and 5.36%, respectively). Similar findings were observed in chronically infected subjects (Figure 5B). Together, these results indicate that during primary CMV infection the increased expression of inhibitory receptors by the expanded population

Figure 3. Activated and atypical memory B cells (MBCs) express an effector memory phenotype during primary cytomegalovirus (CMV) infection. The expression of lymph node (CCR7, CD62L, CXCR4, and CXCR5) and tissue (CD11c) homing receptors and of surface immunoglobulin G was measured on naive, classical memory, activated memory, and atypical MBCs from pregnant women with primary CMV infection (n = 6–14 per phenotype). Results are expressed as mean fluorescence intensity (MFI) or percentage of positive cells within the B-cell subsets in individual subjects, using the following color code: grey, naive B cells; blue, classical MBCs; red, activated MBCs; and green, atypical MBCs. Bars indicate median MFI or median percentage of positive cells. *P < .05; **P < .001; ***P < .0001. Abbreviation: BC, B cell.
of atypical MBCs is associated with relatively low levels of markers of activation and with a relatively low response to polyclonal activators.

**Activated and Atypical Memory B Cells Are Enriched in CMV-Specific Cells**

To determine whether the expansion of activated and atypical MBCs during primary CMV infection involved virus-specific cells, B-cell subsets were labeled with fluorescent polypeptides derived from CMV tegument pp150 and pp52 proteins, known to induce a rapid antibody response following primary infection [31], and tetanus toxoid was used as a control antigen. Antigen-specific cell enrichment was assessed by comparing cell frequencies detected in the IgD-negative memory subsets to the background frequencies detected in the naive subset. During primary infection, CMV-specific B cells were detected during primary CMV infection. The expression of inhibitory receptors (CD22, CD72, LAIR1, CD85j, and FCRL4) and of markers of activation (CD80, CD95, and Ki-67) was measured on naive, classical memory, activated memory, and atypical memory B cells from pregnant women with primary CMV infection (n = 12–15 per phenotype). Results are presented as representative flow cytometry histograms (left panels) and expressed as mean fluorescence intensity (MFI) or percentage of positive cells (right panels) within the B-cell subsets of individual subjects using the same color code as in Figure 3. Bars indicate median MFI or median percentage of positive cells. Lines in histograms indicate threshold of positive expression. *P < .05; **P < .001; ***P < .0001. Abbreviation: BC, B cell.
in classical (median, 0.04%), activated (0.30%), and atypical (0.06%) MBCs, and cell frequencies were significantly higher in activated MBCs, compared with classical and atypical MBC (Figure 6B). Similar results were obtained in chronic infection except that CMV-specific cells were detected at lower frequencies in activated MBCs (0.03%). During primary and chronic CMV infection, tetanus toxoid–specific cells were detected at similar frequencies in classical and activated MBCs and at very low frequencies in atypical MBCs. Together, these results indicate that activated and atypical MBCs are enriched in CMV-specific cells during primary and chronic CMV infection and that primary infection is associated with high frequencies of CMV-specific cells within the activated MBC subset.

**DISCUSSION**

This study shows that primary CMV infection induces large and sustained expansions of activated and atypical MBCs. In contrast, chronically infected and seronegative subjects displayed similar frequencies of the 2 subsets, indicating that their expansion does not persist through the chronic phase of CMV infection. During the first months of primary infection, the proportions of immature CD10+ B cells decreased, whereas the proportions of naive B cells increased, suggesting that a large pool of naive B cells differentiates into memory cells during the early phase of CMV infection and is replenished by an increased output of B-cell precursors from the bone marrow. Our study was conducted in pregnant women. Although pregnancy itself did not induce alterations in the proportions of MBC subsets, it may have contributed to the alterations induced by primary CMV infection.

Expansions of activated MBCs have been described in patients chronically infected with HIV, hepatitis C virus (HCV) or *Plasmodium falciparum* [17, 18, 24]. This study indicates that the relatively prolonged viral replication associated with primary CMV infection also induces the activation of a large pool of memory B cells. The expanded subset of activated MBCs had a phenotype of effector cells. Activated MBC also expressed high levels of markers of activation. In addition, a proportion of activated MBCs spontaneously produced TNF-α in vitro. As previously described, the phenotype of activated MBCs was distinct from that of plasma cells [24]. Indeed, plasma cells included very low frequencies of surface IgG+ cells and were CD22 and CD72 negative (data not shown). The antigen specificity of activated MBCs has not been assessed in patients infected with HIV, HCV, or *P. falciparum*. During HIV infection, the expansion of activated MBCs has been suggested to be related to the activation of follicular helper CD4+ T cells [37] and to the production of B-cell–stimulating factors induced by immune activation [19]. In this study, the proportions of activated MBCs were higher in viremic women, and activated MBCs were enriched in CMV-specific cells, supporting a causal relationship between high viral loads and activation of MBCs. B cells specific for the pp150 and pp52 tegument proteins represented 0.3% of the total pool of class-switched activated MBCs. As the CMV genome codes for around 200 proteins [38], it is likely that a large fraction of activated MBCs are CMV specific during primary infection. Although the activation of CMV-specific memory B cells probably favors the production of antiviral antibodies, the role of activated MBCs in the control of CMV dissemination remains unclear. Evidence for a role in the control of viral replication comes from studies of simian

Figure 5. Tumor necrosis factor α (TNF-α) production by B-cell subsets. The production of TNF-α by B-cell subsets was assessed by flow cytometry following in vitro incubation of peripheral blood mononuclear cells with medium alone (NS) or with the combination of CpG, CD40L, and anti-immunoglobulin G/immunoglobulin A/immunoglobulin M (S). A. Percentage of TNF-α–positive cells in B-cell subsets from 6 pregnant women with primary cytomegalovirus (CMV) infection. B. Percentage of TNF-α–positive cells in B-cell subsets from 6 healthy CMV-positive donors. Results are expressed as the percentage of TNF-α–positive cells in individual subjects. Bars indicate the median percentage of TNF-α–positive cells. *P < .05. Abbreviations: BC, B cell; MBC, memory B cell.
immunodeficiency virus (SIV)-infected macaques, showing that the depletion of activated MBCs is associated with decreased SIV-specific antibody responses and with rapid disease progression [39].

Primary CMV infection was also associated with the expansion of atypical MBCs. Similar to activated MBCs, atypical MBCs expressed an effector phenotype. In contrast, atypical MBCs expressed higher levels of several inhibitory receptors controlling B-cell activation, including CD22, CD72, LAIR-1, and CD85j, and expressed lower levels of activation markers, compared with activated MBCs. This phenotype is similar to the one described in patients infected with HIV or with *P. falciparum* [18, 20, 24]. Inhibitory receptors have been shown to regulate the function of atypical MBCs [23]. In this study, we show that atypical MBCs have a reduced capacity to produce TNF-α, compared with classical or activated MBCs, supporting the notion that this subset is functionally regulated. As observed for activated MBCs, higher frequencies of atypical MBCs were observed in viremic women, and this subset was enriched in CMV-specific cells. In addition, atypical MBCs expressed higher levels of inhibitory receptors during primary CMV infection, compared with chronic CMV infection. Together, these results suggest that the intense viral replication associated with primary CMV infection promotes the expansion and the functional regulation of CMV-specific atypical MBCs. We recently reported that primary CMV infection is associated with the functional

Figure 6. Activated and atypical memory B cells (MBCs) are enriched in cytomegalovirus (CMV)–specific cells. The proportions of cells specific for CMV tegument proteins pp150 and pp52 or for tetanus toxoid (TT) were measured by dual fluorescent-antigen labeling and flow cytometry of B-cell subsets from women with primary CMV infection (n = 8–10) and from healthy adults with chronic infection (n = 9). A, Representative dot plots of class-switched immunoglobulin D-negative B-cells stained with fluorescent pp150/pp52 CMV tegument in a seronegative subject and a pregnant woman with primary CMV infection or TT antigens in a TT-immune individual. B, Proportions of CMV- or TT-specific cells within class-switched B-cell subsets. Results are expressed as percentage of positive cells in individual subjects. Bars indicate the median percentage of positive cells.
exhaustion of virus-specific CD4⁺ T lymphocytes [4]. It appears therefore that the control of CMV replication during primary infection may be limited by the functional regulation of both T and B lymphocytes. Interestingly, several features distinguish the atypical MBC population induced by primary CMV infection from the one described in chronic HIV infection. First, atypical MBCs were detected at higher frequencies than activated MBCs in HIV-infected patients [24, 40], whereas activated MBCs were more abundant in primary CMV infection. Second, atypical MBCs expressed low but detectable levels of activation in primary CMV activation, whereas activation markers are virtually undetectable on atypical MBCs from HIV-infected patients. Third, the inhibitory receptor FcRL4 was only moderately expressed by atypical MBCs during primary CMV infection, whereas it is markedly upregulated during HIV infection [24]. These results suggest that chronic HIV infection induces a more intense functional regulation of B cells than primary CMV infection. This difference may be related to differences in the level and duration of antigen exposure, as observed for functionally regulated T lymphocytes [41, 42]. Supporting this notion, the proportion of atypical MBCs was reduced in HIV-infected patients following the initiation of highly active antiretroviral therapy [40]. Evidence that atypical MBCs could play a role in the pathogenesis of other viral infections is limited. No expansion of atypical MBCs was observed in patients with chronic HCV infection [17]. However, persistent expansions were detected in patients coinfected with HCV and HIV who experienced a relapse after type 1 interferon therapy [43].

In conclusion, this study shows that primary CMV infection mobilizes a large pool of memory B cells that includes activated and atypical MBCs. The activation and the functional regulation of CMV-specific MBCs may have an important impact on the production of antibodies, the control of viral dissemination, and the risk of vertical transmission during pregnancy. The role of atypical and activated MBCs in the control of CMV and the potential impact of modulating the activity of these subsets could be investigated in animal models such as the rhesus macaque [44].

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. A. M. has served as a consultant for GlaxoSmithKline Vaccines and for Hookipa Biotech. The Institute for Medical Immunology, which employs N. D., S. L., and A. M., is cofunded by the Walloon Region and GlaxoSmithKline Vaccines. All other authors report no potential conflicts.

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