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Hepatitis A Virus Infection

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J Immunol published online 31 May 2013
http://www.jimmunol.org/content/early/2013/05/31/jimmunol.1203540

Supplementary Material
http://www.jimmunol.org/content/suppl/2013/05/31/jimmunol.1203540.DC1

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Antibody-Secreting Cells with a Phenotype of Ki-67\textsuperscript{low}, CD138\textsuperscript{high}, CD31\textsuperscript{high}, and CD38\textsuperscript{high} Secrete Nonspecific IgM during Primary Hepatitis A Virus Infection

Seokchan Hong,*† Hyun Woong Lee,‡ Dong-Yeop Chang,* Sooseong You,* Jihye Kim,* Jun Yong Park,§ Sang Hoon Ahn,§ Dongeun Yong,§ Kwang-Hyub Han,§ Ook Joon Yoo,† and Eui-Cheol Shin*

Although studies investigating the nature of Ab-secreting cells (ASCs) during acute infection with influenza or dengue virus found that the ASC response was dominated by virus-specific IgG secretion, the Ag specificity and phenotype of ASCs during primary acute viral infection were not identified. To this end, we investigated the nature of ASCs in direct ex vivo assays from patients with acute hepatitis A caused by primary infection with hepatitis A virus (HAV). We found that the frequency of CD27\textsuperscript{high}CD38\textsuperscript{high} ASCs was markedly increased in the peripheral blood during the acute phase of HAV infection. Moreover, substantial numbers of ASCs were non-HAV–specific and dominantly secreted IgM. We detected HAV-specific ASCs by staining with fluorochrome-tagged HAV-VP1 protein. As compared with HAV-specific ASCs, non-HAV–specific ASCs were Ki-67\textsuperscript{low}CD138\textsuperscript{high}CD31\textsuperscript{high}CD38\textsuperscript{high}, demonstrating that non-HAV–specific ASCs had a bone marrow plasma cell–like phenotype whereas HAV-specific ASCs had a phenotype typical of circulating plasmablasts. These data suggest that non-HAV–specific ASCs might be mobilized plasma cells from the bone marrow or the spleen, whereas HAV-specific ASCs were newly generated plasmablasts. In this study, we provide evidence that pre-existing plasma cells are released into the circulation and contribute to Ag-nonspecific secretion of IgM during primary HAV infection. 

The Journal of Immunology, 2013, 191: 000–000.

Background: After primary viral infection, naive B cells differentiate into short-lived, IgM-secreting plasmablasts in the extrafollicular foci of secondary lymphoid organs, thus contributing to the first line of defense (1). With the help of T cells, some of the B cells continue to proliferate and form germinal centers, where they undergo affinity maturation and isotype switching (1). Following the germinal center reaction, plasma cells are generated and provide protective immunity against virus through the production of neutralizing Abs. In the B cell maturation process, plasma cells are terminally differentiated effector cells and reside in the bone marrow (BM) where they can survive for an extended period of time (2).

Recent studies have evaluated the Ag specificity and phenotype of Ab-secreting cells (ASCs) in subjects who received booster vaccinations of tetanus toxoid (TT) (3, 4). These studies observed a peak in the frequency of ASCs at days 6–7 after boosting and detected not only immunogen-specific ASCs, but also Ag-nonspecific ASCs (3, 4). Intriguingly, the immunogen-specific and non-specific ASCs displayed different phenotypes, with the proliferation marker Ki-67 being highly expressed by immunogen-specific ASCs (4). In contrast, nonspecific ASCs expressed higher levels of the chemokine receptors CXCR3 and CXCR4 than did immunogen-specific ASCs (3, 4). According to their phenotypes, it was proposed that Ag-specific ASCs are newly generated plasmablasts and that nonspecific ASCs are mobilized plasma cells from BM (3–5).

Although ASCs have not been studied in detail during human primary viral infection, they have been studied during possible reinfection with influenza and dengue virus (6, 7). These studies showed remarkable increases in the numbers of ASCs in the circulation during acute infection with influenza virus or dengue virus, and these cells dominantly secreted virus-specific IgG (6, 7). Given that influenza and dengue viruses often infect previously infected persons, infection with these viruses likely does not represent primary viral infection; thus, the ASCs could have been derived from pre-existing memory cells (6–8). Studies of ASCs in primary dengue virus infection have been conducted. However, these studies evaluated ASCs by means of in vitro stimulation and not by direct ex vivo analyses and, therefore, they did not directly examine ASCs (9, 10). Additionally, the Ag specificity and phe-
notype of ASCs have not been studied during primary acute viral infection (8).

Hepatitis A virus (HAV) is a small, positive-stranded and non-enveloped RNA virus (11). Primary infection with HAV often results in symptomatic acute hepatitis in adults (11), and HAV infection or vaccination induces life-long protective immunity through the production of neutralizing Abs (12). Given the life-long protective immunity against HAV, acute hepatitis A (AHA) could serve as a proper model for the study of primary acute viral infection in humans.

In the present study, we investigated the Ag specificity and phenotypes of circulating ASCs in AHA patients. The frequency of CD27hiCD38hi ASCs was greatly increased in the peripheral blood during the acute phase of HAV infection, and these cells dominantly secreted IgM. A substantial fraction of the secreted IgM Abs was reactive to non-HAV Ags, suggesting an Ag-nonspecific response of ASCs. Through phenotype analysis, non-HAV–specific ASCs were found to be Ki-67lowCD138hiCD31hiCD38hi compared with HAV-specific ASCs, demonstrating that non-HAV–specific ASCs had a BM plasma cell-like phenotype whereas HAV-specific ASCs had a phenotype typical of circulating plasmablasts (8, 13, 14). These data suggest that non-HAV–specific ASCs are plasma cells originating from the BM or the spleen, whereas HAV-specific ASCs are newly generated plasmablasts. Thus, in this study we show that during primary infection by HAV, pre-existing plasma cells are released into the circulation and contribute to Ag-nonspecific secretion of IgM.

Materials and Methods

Patients and blood samples

The study included 39 patients (age range, 19–41 y; median age, 34 y) diagnosed with AHA who were hospitalized at Chung-Ang University Hospital (Seoul, Korea) or at Severance Hospital (Seoul, Korea). All patients were seropositive for anti-HAV IgM, with or without IgG, and all had features of acute hepatitis based on clinical and laboratory findings. Peripheral blood samples at the acute phase were collected in EDTA tubes and serum separator tubes on the day of admission from all 39 patients. Follow-up sampling was performed during the subacute phase (5–14 d after admission) or during the convalescent phase (35–150 d after admission) in some of the patients (22 patients for the subacute phase and 16 patients for the convalescent phase). PBMCs were isolated from EDTA tubes by density-gradient centrifugation over Ficoll-Paque (GE Healthcare, Piscataway, NJ) and then cryopreserved. Serum was separated from serum separator tubes. This study was approved by the Institutional Review Boards of Chung-Ang University Hospital and Severance Hospital.

Reagents

Purified HAV particles were purchased from Microbiobiosystems (Toronto, ON, Canada) and Fitzgerald Industries (Concord, MA) and were used for the ELISA and ELISPOT assays, respectively. Recombinant HAV VP1-P2A protein was obtained from ViroGen (Watertown, MA) and was conjugated with Alexa Fluor 647 using a mAb labeling kit (Molecular Probes, Eugene, OR). The following proteins were used in the ELISA and/or the ELISPOT assays: TT (Calbiochem, La Jolla, CA) and diaphragm toxoid (DT; List Biological Laboratories, Campbell, CA).

ELISA and cytometric bead array assays

MaxiSorp microtiter plates (Nunc, Roskilde, Denmark) were coated with Ags at 4°C. After blocking with 5% BSA, diluted serum was added to the plates and incubated overnight at 4°C. Biotin-conjugated donkey anti-human IgM or IgG (Jackson ImmunoResearch, West Grove, PA) was then added, followed by incubation with streptavidin-HRP. Tetramethylbenzidine (SureBlue; KPL, Gaithersburg, MD) was used as a substrate for the HRP. The colorimetric reaction was stopped with 2 M H2SO4, and the absorbance was measured at an OD of 450 nm (with the reference wavelength of 655 nm) on a microplate reader (Bio-Rad Laboratories, Hercules, CA). The levels of IgM and IgG specific for measles and rubella were measured by commercially available ELISA kits (Abnova, Taipei, Taiwan). The level of IgG specific for TT was also measured by a commercially available ELISA kit (Abcam, Cambridge, MA).

Serum levels of the total IgM, IgG, and the subtypes of IgG were measured by cytometric bead array assay (human Ig flex set; BD Biosciences, San Jose, CA).

ASC ELISPOT assay

ELISPOT filter plates (Millipore, Bedford, MA) were coated overnight with anti-human Ig to detect the total IgM- or IgG-secreting ASCs. For Ag-specific ASCs, plates were coated with an HAV particle, TT, or DT. The coated plates were blocked for 2 h at 37°C with RPMI 1640 supplemented with 0.1% OVA. After blocking, 10,000–20,000 PBMCs were added to anti-human Ig-coated wells, and 2.0–2.5 ¥ 105 PBMCs were added to Ag-coated wells. The plates were incubated at 37°C overnight. After washing with PBS and 0.05% Tween 20–PBS, the plates were incubated with either biotin-conjugated anti-human IgM or with anti-human IgG for 2 h at room temperature. Then, streptavidin–alkaline phosphatase (Caltag, South San Francisco, CA) was added and incubated for 1 h. The colorimetric reaction was performed with the AP color development reagent (Bio-Rad Laboratories). Spots were counted using an ELISPOT reader (Cellular Technologies, Cleveland, OH), and the number of specific spots was calculated as the number of spots in Ag-coated well minus the number of spots in control well without Ag coating.

Flow cytometric analysis

Cryopreserved PBMCs were thawed and incubated with FcR blocking reagent (Miltenyi Biotec, Auburn, CA) to prevent FcR-mediated Ab binding, after which cell surface staining was performed for 30 min at 4°C using combinations of the following mAbs: CD3-PerCP-Cy5.5, CD10-PE, CD14-PerCP-Cy5.5, CD19-Alexa Fluor 700 or PE, CD27-allophycocyanin-H7, CD31-PE, CD38-PE-Cy7, CD56-PerCP-Cy5.5, CD138-FITC, IgD-FITC (BD Biosciences); CD19-Pacific Blue, CD20-Pacific Blue or eFluor 450 (eBioscience, San Diego, CA); and CXCR3-PE (R&D Systems, Minneapolis, MN). Dead cell staining was performed using ethidium monoazide (Molecular Probes). For intracellular staining, cells were fixed and permeabilized using BD Cytofix/Cytoperm and BD Perm/Wash (BD Biosciences). The intracellular protein was then stained using the following mAbs: IgM-allophycocyanin or FITC, IgG-PE, Ki-67-FITC, or PE-Cy7 (BD Biosciences). To detect HAV-VP1–specific ASCs, PBMCs were permeabilized and stained with Alexa Fluor 647–conjugated HAV-VP1 (2 μg/ml) for 30 min at room temperature. Immunostained cells were acquired on an LSR II instrument (BD Biosciences) and the data were analyzed using FlowJo software (Tree Star, Ashland, OR).

Statistical analysis

Statistical analyses were performed with Prism 5 software (GraphPad Software, San Diego, CA). Comparisons of B cell frequencies or Ab titers between groups were performed using unpaired two-tailed t tests. Temporal changes of the parameters between serial measurements were analyzed by two-tailed paired t tests. To assess the differences between HAV-VP1–specific and non-HAV-VP1–specific ASCs, Wilcoxon signed-rank tests were performed.

Results

The frequency of CD27hiCD38hi ASCs was increased during the acute phase of HAV

We studied whether the frequency of ASCs is increased in the peripheral blood during the acute phase (day of patient admission to the hospital) of AHA, which is caused by primary infection with HAV. Using multicolor flow cytometry, we identified CD19+ circulating ASCs by their high levels of expression of CD27 and CD38 without CD10 expression (Fig. 1A). A robust increase of CD19+CD27hiCD38hi, confirming the phenotype of the ASCs (Fig. 1B). Statistical analyses demonstrated a significant increase in ASCs in the peripheral blood during the acute phase without a change in the frequency of total CD19+B cells (Fig. 1C).

In this regard, the increased frequency of ASCs was maintained until the subacute phase (5–14 d after admission) and significantly decreased during the convalescence phase (35–150 d after admission) (Fig. 1D). Notably, the frequency of naive B cells was significantly decreased during the acute phase, whereas the frequencies of im-
mature B cells and memory B cells remained unchanged (Fig. 1C). Taken together, our results demonstrate that there is a robust increase of CD27highCD38high ASCs in the peripheral blood of AHA patients. ASCs dominantly secrete IgM during AHA

To address which isotypes of Ab were produced by these ASCs, we stained these cells intracellularly for IgM and IgG during the acute phase of AHA (Fig. 2A) and found that the frequency of IgM+ cells was significantly higher than that of IgG+ cells (Fig. 2B). The IgM-dominant response was confirmed by direct ex vivo ASC ELISPOT assay to quantify the total numbers of IgM- and IgG-secreting cells. During the acute phase, the peripheral blood of AHA patients had a significantly higher ratio of IgM ASCs to IgG ASCs compared with that of healthy controls, whereas this ratio was significantly decreased during the convalescence phase (Fig. 2C). We also prospectively determined total amounts of IgM and IgG in patient sera. Total IgM was significantly increased at all three phases of AHA compared with healthy controls (Fig. 2D). In particular, total IgM levels at the acute and subacute phases were strikingly increased (Fig. 2D). In contrast, total IgG was only slightly increased during the subacute phase compared with healthy controls (Fig. 2E), and there were no significant changes in the concentrations of the IgG subclasses such as IgG2, IgG3, and IgG4 (Supplemental Fig. 1). Taken together, these data demonstrate that in AHA patients the Ab-secreting response is dominated by IgM secretion.

FIGURE 1. ASCs in the peripheral blood during the acute phase of AHA. (A) In PBMCs, CD19+ B cells were defined as CD19+CD3−CD14−CD56−ethidium monoazide−cells, and in the gating of CD19+ B cells, ASCs were identified as CD10−CD27highCD38high cells. (B) In the gating of CD19+ B cells, the presence of ASCs was confirmed by the phenotype CD20−CD38high or IgD−CD3high. Representative flow cytometry plots are presented. (C) The frequency of CD19+ B cells in the gating of PBMCs and frequencies of ASCs, naive B cells, immature B cells, and memory B cells in the gating of CD19+ B cells were analyzed and compared between the acute phase PBMCs of AHA patients (n = 39) and PBMCs of healthy controls (n = 16). Each subset of B cells was defined as follows: ASCs, CD10−CD27highCD38high; naive B cells, CD10+CD27−CD38−IgD+; immature B cells, CD10−CD27−CD38high; and memory B cells, CD10−IgD−CD38−CD27+. (D) The frequency of ASCs in PBMCs was analyzed at the indicated phase of AHA and compared by paired t test between the acute and subacute phases (left, n = 22) or between the acute and convalescence phases (right, n = 13). *p < 0.05, **p < 0.01, ***p < 0.001.
ample of non-HAV–specific IgM and obtained similar results (Supplementary Fig. 2). In contrast, AHA patients presented HAV-specific IgG (Fig. 3C) without an increase in non-HAV–specific IgG (Fig. 3D). As expected, the HAV-specific IgG titer reached its peak level during the convalescence phase.

We further evaluated the Ag specificity of IgM ASCs in AHA patients by direct ex vivo ELISPOT assays and determined the number of HAV-, TT-, or DT-specific spots. In PBMCs obtained during the acute phase, we detected not only HAV-specific IgM ASCs, but also TT- and DT-specific IgM ASCs. These IgM ASCs disappeared during the convalescence phase (Fig. 3E). Intriguingly, the frequency of HAV-specific IgM ASCs was ~2.5% of the total IgM ASCs, a relatively small proportion out of the total IgM ASCs (Fig. 3F). As we unexpectedly observed expansion of non-HAV–specific IgM and obtained similar results (Supplemental Fig. 2). In contrast, AHA patients presented HAV-specific IgG (Fig. 3C) without an increase in non-HAV–specific IgG (Fig. 3D). As expected, the HAV-specific IgG titer reached its peak level during the convalescence phase.

We further evaluated the Ag specificity of IgM ASCs in AHA patients by direct ex vivo ELISPOT assays and determined the number of HAV-, TT-, or DT-specific spots. In PBMCs obtained during the acute phase, we detected not only HAV-specific IgM ASCs, but also TT- and DT-specific IgM ASCs. These IgM ASCs disappeared during the convalescence phase (Fig. 3E). Intriguingly, the frequency of HAV-specific IgM ASCs was ~2.5% of the total IgM ASCs, a relatively small proportion out of the total IgM ASCs (Fig. 3F). Similar numbers of IgM ASCs secreted TT- or DT-specific IgM (Fig. 3E, 3F), demonstrating that a significant percentage of IgM ASCs secrete non-HAV–specific IgM.

Non-HAV–specific ASCs are phenotypically different from HAV-specific ASCs

As we unexpectedly observed expansion of non-HAV–specific ASCs in the peripheral blood of AHA patients, we next used flow cytometric analysis to determine whether non-HAV–specific ASCs are phenotypically different from HAV-specific ASCs to identify the possible origin of these populations. We successfully identified HAV-specific ASCs by cell permeabilization and staining with fluorochrome-tagged HAV-VP1, an immunodominant protein (Fig. 4A) (15). HAV-VP1–stained cells were observed in the CD38high ASC fraction during the acute phase of AHA, but not in healthy controls (Fig. 4A). Specific binding of fluorochrome-tagged HAV-VP1 was confirmed by competition with an excess amount of unconjugated HAV-VP1 (Fig. 4B), or by blocking with serum from HAV-immune individuals (Fig. 4C).

By staining with fluorochrome-tagged HAV-VP1, HAV-VP1–specific ASCs could be distinguished from non-HAV-VP1–specific ASCs, and their phenotypes were further compared (Fig. 4D). Approximately 11% of ASCs were bound to HAV-VP1 during the acute phase of AHA (Fig. 4E). Both HAV-VP1–specific and non-HAV-VP1–specific ASCs were dominantly IgM+ rather than IgG+ in the peripheral blood during the acute phase of AHA (Supplemental Fig. 3). Ki-67, a proliferation marker, and the BM homing markers CD138 and CD31 were compared between non-HAV-VP1–specific and HAV-VP1–specific ASC populations. Non-HAV-VP1–specific ASCs were found to express significantly lower levels of Ki-67 but higher levels of CD138 and CD31 than HAV-VP1–specific ASCs (Fig. 4F, 4G). This phenotype of Ki-67lowCD138highCD31high non-HAV-VP1–specific ASCs was compatible with BM plasma cells rather than circulating plasmablasts (8, 14). We also analyzed CD38, which is...
overexpressed in BM plasma cells compared with circulating plasmablasts (8, 16). Non-HAV-VP1–specific ASCs expressed significantly higher levels of CD38 than did HAV-VP1–specific ASCs (Fig. 4F, 4G).

Additionally, we stained DT-specific ASCs and examined their phenotype as an example of non-HAV–specific ASCs. DT-specific ASCs were successfully detected in the peripheral blood during the acute phase of AHA (Supplemental Fig. 4A), and they expressed significantly lower levels of Ki-67 than did HAV-VP1–specific ASCs (Supplemental Fig. 4B). Expression level of Ki-67 in DT-specific ASCs was comparable to that in unstained HAV-VP1 ASCs (Supplemental Fig. 4B). These data show that un-
stained HA V-VP1 ASCs can be considered non-HA V–specific ASCs in the phenotype analysis. In summary, non-HA V–specific ASCs are Ki-67lowCD138highCD31highCD38high compared with HAV-specific ASCs (Table I). This demonstrates that non-HAV–specific ASCs have a BM plasma cell–like phenotype whereas HAV-specific ASCs are phenotypically similar to circulating plasmablasts. Taken together, these data suggest that non-HAV–specific ASCs are mobilized plasma cells, whereas HAV-specific ASCs are newly generated plasmablasts. Additionally, the non-HAV–specific ASCs were also CXCR3high (Fig. 4H), further consistent with mobilized plasma cells (3).

Discussion
In the present study we conducted a detailed characterization of ASCs during AHA. During the acute phase, the frequency of stained HA V-VP1 ASCs can be considered non-HA V–specific ASCs in the phenotype analysis. In summary, non-HAV–specific ASCs are Ki-67lowCD138highCD31highCD38high compared with HAV-specific ASCs (Table I). This demonstrates that non-HAV–specific ASCs have a BM plasma cell–like phenotype whereas HAV-specific ASCs are phenotypically similar to circulating plasmablasts. Taken together, these data suggest that non-HAV–specific ASCs are mobilized plasma cells, whereas HAV-specific ASCs are newly generated plasmablasts. Additionally, the non-HAV–specific ASCs were also CXCR3high (Fig. 4H), further consistent with mobilized plasma cells (3).

**Table I. Phenotypic differences between non-HAV–specific and HAV-specific ASCs in AHA**

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<th>Marker</th>
<th>Non-HAV–Specific</th>
<th>HAV-Specific</th>
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+ Weak expression; ++, middle expression; ++++, strong expression.
consistent with a previous study (17). The increase in IgM-secreting ASCs is strikingly different from the results of previous studies in adults infected by influenza or dengue virus (6, 7). These studies showed marked increases in ASC numbers dominated by virus-specific IgG secretion (6, 7). The differences between our studies and others may be explained by the unique nature of different viral infections. Adult populations usually have previously encountered influenza virus, as is likely the case with dengue virus in endemic areas. Additionally, influenza and dengue viruses often reinfect previously infected persons (18), and therefore in adults these viral infections are not likely primary infections. Indeed, serological data and the memory B cell responses seen in these studies revealed that patients had a previous history of exposure to the virus (6, 7). In contrast, HAV infection leads to lifelong protective immunity that efficiently prevents reinfection by the virus (11). Because HAV results from the primary infection of HAV, we studied ASCs in this model of primary viral infection of humans. In particular, ASCs were studied by direct ex vivo assays in the present study rather than through in vitro stimulation (9, 10).

Additionally, we studied the Ag specificity of the secreted IgM and ASCs in AHA patients. First, we found that the increased serum IgM at the acute and subacute phases had reactivity against not only HAV (Fig. 3A), but also against other unrelated Ags such as TT; measles, and rubella (Fig. 3B). Serum IgM against gut bacteria such as Bacteroides fragilis, Escherichia coli, and Enterococcus faecalis was also increased at the acute and subacute phases (Supplemental Fig. 2), as previously reported during the acute phase of AHA (19). Additionally, we studied the Ag specificity of IgM ASCs by direct ex vivo ELISPOT assay. We confirmed the serological data by showing that PBMCs during the acute phase included not only HAV-specific IgM ASCs, but also TT- or DT-specific IgM ASCs (Fig. 3E). In fact, the HAV-specific IgM ASCs represented a relatively small proportion (~2.5%) of the total IgM ASCs (Fig. 3F). Cells secreting TT- or DT-specific IgM accounted for ~2% of the total IgM ASCs, confirming that substantial numbers of IgM ASCs secreted non-HAV-specific IgM (Fig. 3F). Of note, PBMCs isolated during the acute phase yielded substantial numbers of spots even without Ag coating in control wells, and these nonspecific spots were not observed in PBMCs isolated from patients during the convalescence phase of AHA (data not shown). The background spots by the acute phase PBMCs might be related to OVA, which was included in blocking solution. The similar background spots were also observed when BSA was used for blocking solution instead of OVA. These findings further confirm Ag nonspecificity of ASCs during the acute phase of AHA. The Ag-nonspecific response during AHA stands in contrast with the Ag-specific response during influenza or dengue viral infection. This difference may result from the unique nature of each viral infection in the study population; that is, primary infection of HAV versus reinfection of influenza or dengue viruses. Further studies are needed to address whether the Ag-nonspecific ASCs are induced upon primary acute infection of other viruses.

In this study, we analyzed the phenotypes of non-HAV- and HAV-specific ASCs and explored a possible origin of non-HAV-specific circulating ASCs. We identified HAV-specific ASCs by staining with fluorochrome-tagged HAV-VP1, which is known to be an immunodominant nucleocapsid protein (Fig. 4A) (15). This analysis enabled us to identify the differences in the phenotypes between non-HAV- and HAV-specific ASCs. As a result, non-HAV-specific ASCs were found to be Ki-67highCD138highCD31highCD38high (Table I). Because we identified HAV-specific ASCs by staining with HAV-VP1, which is one of the nucleocapsid proteins, we could not exclude the possibility that unstained HAV-VP1 cells contain HAV-specific ASCs reactive to other HAV proteins. If it had been possible to stain all of the HAV-specific ASCs using multiple HAV proteins in addition to VP1, the difference in phenotype between non-HAV- and HAV-specific ASCs would likely be even greater. We also stained DT-specific ASCs and examined their phenotype as an example of non-HAV-specific ASCs. DT-specific ASCs expressed significantly lower levels of Ki-67 than did HAV-VP1–specific ASCs (Supplemental Fig. 4B). Expression level of Ki-67 in DT-specific ASCs was comparable to that in unstained HAV-VP1 ASCs (Supplemental Fig. 4B). These data show that unstained HAV-VP1 ASCs can be considered non-HAV–specific ASCs in the phenotype analysis.

Previous studies showed that BM plasma cells have a phenotype unique from that of circulating plasmablasts (8, 13, 14, 16, 20). BM plasma cells expressed higher levels of CD138, CD31, and CD38 than did circulating plasmablasts. CD138, a molecule that binds APRIL, is known to be involved in the homing of plasma cells into the BM, whereas the acquisition of CD138 expression is associated with plasma cell differentiation, and is thus used as a plasma cell marker (21, 22). Meanwhile, circulating plasmablasts have a high proliferative capacity, as shown by the higher expression of Ki-67 (20). Therefore, the phenotype of Ki-67lowCD138highCD31highCD38high in the non-HAV–specific ASCs demonstrated that they had a BM plasma cell–like phenotype, whereas HAV-specific ASCs had the typical phenotype of circulating plasmablasts. These data suggest that non-HAV–specific ASCs were mobilized plasma cells from the niches of plasma cells, whereas HAV-specific ASCs were newly generated plasmablasts. Compared to HAV-specific ASCs, in fact, non-HAV–specific ASCs presented a phenotype of CXCR3high (Fig. 4H), which is the phenotype of ASCs mobilized from BM plasma cells (3).

As a source of non-HAV–specific ASCs, we need to consider not only the BM but also the spleen. It was demonstrated that long-lived plasma cells were detected in human spleen, and that these cells exhibited characteristics of BM plasma cells including high expression of CD31 (23). Interestingly, these splenic plasma cells dominantly secrete IgM, whereas BM plasma cells secrete IgG. Therefore, it is appealing to hypothesize that non-HAV–specific IgM+ ASCs might be mobilized plasma cells from the spleen during the acute phase of AHA. The mechanisms of mobilization of BM or splenic IgM+ plasma cells to the peripheral blood during the acute phase of AHA are currently under investigation.

In summary, we demonstrated that in AHA, the frequency of CD27highCD38high ASCs is vigorously increased in the peripheral blood, with most of these cells producing Ag-nonspecific IgM. Whereas HAV-specific ASCs exhibited the phenotype typical of circulating plasmablasts, non-HAV–specific ASCs exhibited the phenotype of BM or splenic plasma cells. These data suggest that non-HAV–specific ASCs are plasma cells originating from the BM or the spleen, whereas HAV-specific ASCs are newly generated plasmablasts. Taken together, we proposed that pre-existing plasma cells are released into the circulation during AHA and contribute to Ag-nonspecific secretion of IgM.

Acknowledgments
We thank Wonseok Kang, Seung Joo Nam, Jino Lee, and Yoon Seok Choi (Laboratory of Immunology and Infectious Diseases, Korea Advanced Institute of Science and Technology) for critical review of this manuscript.

Disclosures
The authors have no financial conflicts of interest.
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