Bone Marrow Plasma Cells Dictate Serum HIV-Specific Antibodies in Chronic Viremia

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Abstract

Background: Screening for HIV-specific antibody neutralizing activity in the serum or plasma has been the starting point for the isolation of several potent and broadly neutralizing antibodies to HIV from peripheral blood B cells of infected individuals. Despite the success of this approach, little is known regarding the cells that produce the antibodies detected in the serum. To gain a better understanding of the cellular source of circulating antibodies, we investigated two possible sources, the bone marrow and peripheral blood, of chronically HIV-viremic individuals.
Methods: Bone marrow biopsies and peripheral blood samples were obtained from eight chronically HIV-viremic individuals and bone marrow cells were obtained from eight healthy HIV-uninfected controls. Written informed consent was provided. Immunophenotyping was performed on cell suspensions using B-cell subset-defining markers. A biotinylated HIV envelope gp140 probe was used to measure HIV-specific antibodies in serum (via ELISA), HIV-specific plasmablasts (via ELISPOT) or memory B cells (via flow cytometry) in blood, and HIV-specific plasma cells in bone marrow (via ELISPOT).
Results: Compared to HIV-uninfected counterparts, the bone marrow aspirates of the HIV-infected participants contained increased frequencies of plasma cells and B cell precursors (namely preB-I and preB-II), and decreased frequencies of mature B cells. Levels of Env-specific antibodies measured in serum were compared to corresponding frequencies of antibody-secreting or -binding cells measured in the bone marrow (plasma cells) and the blood (plasmablasts and memory B cells). A strong correlation was observed between HIV-specific antibodies in serum and the Env-specific bone marrow-derived plasma cells, but not the plasmablasts or memory B cells from blood.
Conclusions: Increased frequencies of plasma cells in the bone marrow are consistent with known hallmarks of HIV infection, namely hypergammaglobulinemia and increased frequencies of peripheral blood plasmablasts. These findings demonstrate that despite HIV-induced phenotypic and functional B-cell dysregulation in the peripheral blood and secondary lymphoid tissues, bone marrow plasma cells remain a primary source for circulating Env-specific antibodies in HIV-infected individuals.

Background and Rationale

- Several HIV envelope (Env)-specific monoclonal antibodies have recently been characterized in infected individuals (1-5); however, little is known regarding the association between serum antibodies and the cellular and tissue sources from which they originate.
- Potential cellular origins include plasmablasts (PBs) and plasma cells (PCs) that are continuously secreting antibodies or memory B cells that can secrete antibodies upon terminal differentiation (6-10).
- Tissue origins include bone marrow and secondary lymphoid tissues, such as spleen, lymph nodes and mucosal lymphoid sites (6-8).
- We evaluated Env-specific B cell responses in two different compartments of HIV-infected chronically viremic individuals, namely bone marrow and peripheral blood, and correlated these responses with circulating Env-specific antibodies.

Table 1

Demographic and immunologic profiles of HIV-1-infected individuals

Patient number	Age (y)	Gender	Ethnicity	Estimated[a] time of infection at biopsy[b] (months)	Plasma HIV-1 RNA at time of biopsy[b] (copies/ml)	CD4 count at time of biopsy (cells/μl)	Serum Env binding ELISA (OD)
1	26	Male	Hispanic	14	1,118	777	0.428
2	34	Male	Hispanic	16-28	14,200	460	0.816
3	31	Male	Black	>120	164	714	2.461
4	26	Female	Black	19	2,549	479	0.95
5	44	Male	White	>96	3,739	403	2.593
6	29	Female	Hispanic	>101	1,562	434	2.091
7	28	Male	Hispanic	27	20,964	397	3.189
8	23	Male	Hispanic	23	19,156	875	2.989

[a]Estimated from clinical history taken at screening visit.
[b]All were ART-naive, except #5 and #6 discontinued ART 35 and 75 months prior to study, respectively.

References

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Results

Figure 1

Phenotypic characterization of bone marrow B cells and subsets, based on predicted maturation pattern and expression of other distinctive markers.

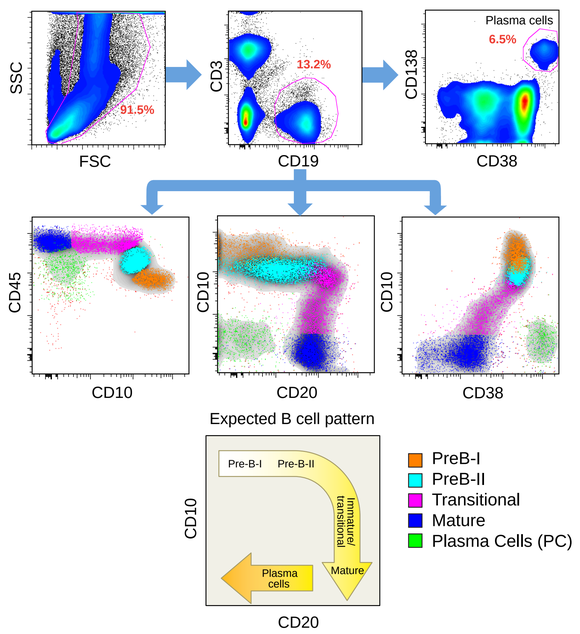


Figure 2

Bone marrows of HIV-infected individuals contain higher frequencies of PreB-I, PreB-II and plasma cells (aspirate and core biopsy) and lower frequencies of mature B cells than HIV-uninfected individuals.

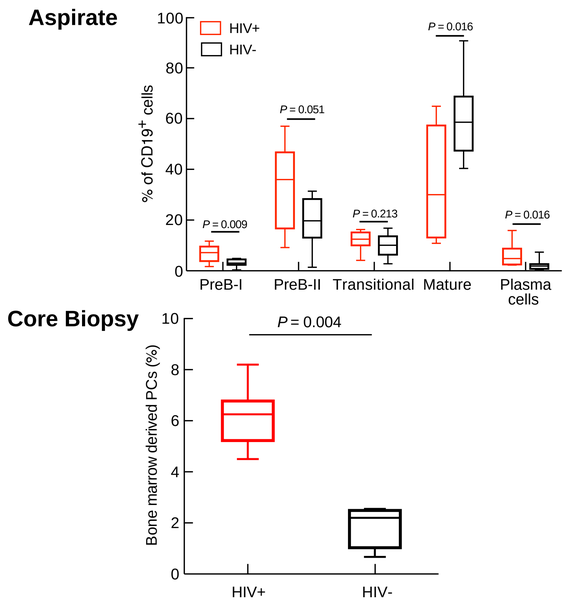


Figure 3

Frequencies of total and Env-specific antibody-secreting cells (ASCs) in the bone marrow of HIV-infected individuals.

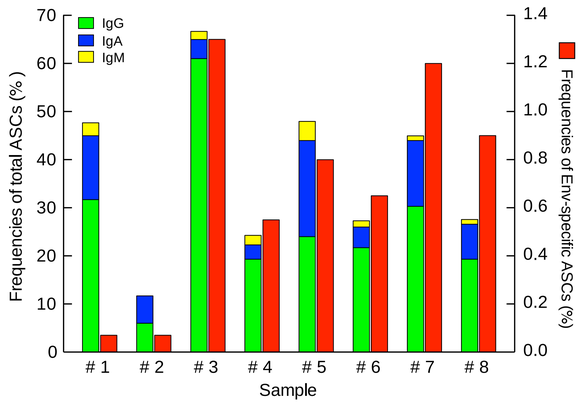


Figure 4

Frequencies of total and Env-specific antibody-secreting cells (ASCs) and Env-specific memory B cells in the peripheral blood of HIV-infected individuals.

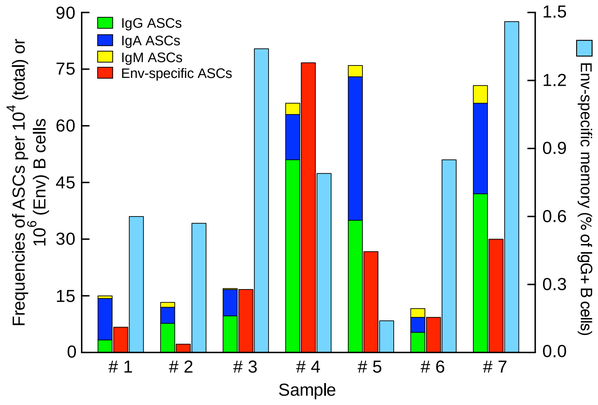
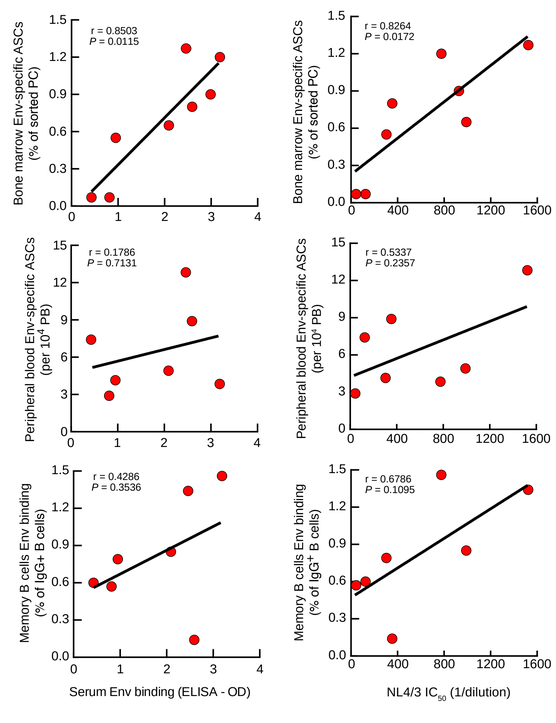


Figure 5

Strong significant correlation between Env-binding (left panels) and HIV-neutralizing (right panels) antibodies in serum and Env-specific plasma cells in the bone marrow of HIV-infected individuals.



Conclusions

- In HIV-infected chronically viremic individuals, levels of circulating HIV Env-specific antibodies strongly correlated with frequencies of Env-specific bone marrow-derived PCs, whereas there was no correlation with peripheral blood Env-specific PBs or memory B cells. This finding demonstrates that bone marrow-derived PCs are the main source of HIV-specific antibodies in serum even in the setting of chronic HIV viremia.
- Frequencies of B-cell precursors and plasma cells were elevated in the bone marrow from HIV-infected individuals, while those of mature B cells were reduced. This finding may indicate homeostatic dysregulation caused by chronic HIV infection.
- Given that bone marrow PCs are likely the durable repository of antibodies responsible for maintaining long-term humoral immunity, this compartment should be included as part of the evaluation of responses to immunogens being considered as HIV vaccine candidates.

Materials and Methods

Study Participants: Bone marrow aspirates and core biopsies were obtained from HIV-1-infected individuals (Table 1). Samples from HIV-uninfected individuals were obtained from the Department of Laboratory Medicine, Clinical Research Center, NIH. Bone marrow core biopsies and aspirates were obtained from posterior iliac crests under conscious sedation and local anesthesia. All study subjects provided informed consent, in accordance with the Institutional Review Board of the National Institute of Allergy and Infectious Diseases or Clinical Center, National Institutes of Health.

Morphologic analyses: Sections from decalcified formalin-fixed, paraffin-embedded core bone marrow biopsies were stained with CD138 antibody using an autostaining system (Ventana). Immunohistochemistry stains were digitally scanned and analyzed to determine the percentage of $CD138^+$ cells in the specimens.

Processing of bone marrow aspirates and peripheral blood: Three serial bone marrow aspirate pulls were obtained from each subject. Quality assessment of each pull was performed by establishing their hemodilution, based on the presence of mature neutrophils ($CD13^+/CD16^+/CD45^+$). Bone marrow (BMMCs) and peripheral blood mononuclear cells (PBMCs) were isolated by by Ficoll-Hypaque density gradient centrifugation. BMMCs corresponding to the pull with the lowest hemodilution were used for further processing and analyses. $CD38^{hi}/CD138^+$ PCs were sorted from BMMCs using a FACSAria cell sorter (BD Biosciences). For functional and certain phenotyping analyses, mature ($CD10^-$) B cells were enriched from PBMCs by negative selection.

Phenotypic analyses: Neutrophil staining for analysis of hemodilution was performed by staining for CD13, CD16 and CD45 expression. Freshly processed and cryopreserved BMMCs were used for HIV-infected and HIV-uninfected stains, respectively. Cryopreserved PBMCs were used to measure the frequency of PBs among HIV-infected individuals and the frequency of Env-specific memory B cells. Cell surface phenotyping was performed by staining for CD3, CD19, CD27, CD38, CD10, CD20, CD138 and IgG expression. For Ig isotype (IgM, IgA and IgG) staining of PBs and PCs, cells were permeabilized for intracellular staining. Frequencies of HIV Env-specific memory B cells were measured with a trimeric HIV Env gp140 probe (YU2-gp140-F), as described (9). Flow cytometry was performed on a FACSCanto flow cytometer, with data analyses on FlowJo Version 9.6.

Functional analyses: Frequencies of PBs and PCs secreting IgG, IgA and IgM, as well as those specific for Env, were measured by ELISPOT, as described (10). Briefly, plates were coated with 5 μg/ml anti-Ig light-chain antibodies, followed by addition of sorted plasma cells or PBMC-derived B cells, incubation overnight, and detection with biotinylated antibodies against each of the Ig classes or biotinylated HIV Env (YU2-gp140-F). Unlabeled or biotinyated KLH served as negative control antigen.

ELISA: Plates were coated with 2.5μg/ml goat anti-human IgG (H+L) in PBS overnight at 4°C. Serum samples from study participants and an HIV-negative control were diluted 5-fold, starting from a 1:50 dilution in PBS-0.02% azide, mixed 1:1 with blocking buffer and added to the plate. Consecutive incubations of 1 h interspaced with vigorous washing (PBS-0.02% Tween 20) were as follows: serum sample mix at room temperature (RT); biotinylated YU2-gp140-F (2.5μg/ml) at 37°C; and streptavidin-HRP at RT. Plates were developed 5 min with TMB peroxidase substrate and stopped with HCl, and the OD was measured at 450nm. An OD of two SD above that of HIV-negative serum was considered positive, and OD values at 1/50 dilution of end-point dilution curves were used in data analyses.

HIV neutralization: Serum samples were tested for HIV-neutralizing activity using a pseudovirus assay with a tier 2 panel developed and performed by Monogram Biosciences (11).