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Simian immunodeficiency virus infection in rhesus macaques induces selective tissue specific B cell defects in double positive CD21+CD27+ memory B cells

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Abstract

B cell dysfunction represents a central feature in HIV infection and pathogenesis. Our recent studies have shown that peripheral and lymphoid double positive CD21+CD27+ B cells were able to become activated and proliferate at higher rates than other B cell subpopulations. Increased proliferation of tonsillar memory B cells were identified compared to other tissues examined. Here, we demonstrate the decreased proliferation of tonsillar memory (CD21+CD27+) B cells during acute SIV infection also suggests that these cells may play an important role in SIV pathogenesis. Our findings demonstrate that SIV infection may induce selective defective responses in specific tissues, by suppressing memory B cell proliferation in tissues.

Keywords

B-cells; Memory; Proliferation; SIV; Rhesus Macaque; BrdU

INTRODUCTION

Virus-induced immune-cell activation is one of the few widely accepted hallmarks of HIV/SIV pathogenesis and disease progression. HIV/SIV infection has been associated with a wide range of B cell defects, including increased frequencies of activated and terminally differentiated B cells expressing low levels of CD21 [1, 2], polyclonal hypergammaglobulinemia and the presence of immature/transitional CD10+ or exhausted CD27 negative B cells in blood [3–7], exhaustion of tissue-like memory (CD20(hi)/CD27(−)/CD21(lo)) B cells [8] and loss of memory B cell populations [9]. Early loss of B cells in spleen, lymph nodes and peripheral blood (PB) [10–13] in SIV and disruption of gut germinal center during acute HIV infection [14] may be an indicator of the early failure of

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adaptive immune responses. Although studies have been performed to understand the role of B cells in SIV pathogenesis by artificially depleting B cells [15, 16], the dynamics and mechanism of B cell loss during acute phase of SIV infection are not well characterized. Our recent studies have shown that double positive (DP) CD21+CD27+ B cells are memory cells that are capable of antibody production by polyclonal activation, and without additional help from T cells [17]. Memory CD21+CD27+ B cells were predominant in all lymphoid tissues except for PB. DP CD21+CD27+ B cells were also able to activate and proliferate at higher rates than other B cell subpopulations [17]. In this study, we examined and compared levels of proliferation of T cells and different B cell subsets in lymphoid tissues to correlate their rates of proliferation with plasma viral load.

This study shows that tonsillar CD21+CD27+ B cells are highly proliferating B cells compared to other tissues examined. Furthermore, these data demonstrate differences in proliferative responses of nonhuman primate B memory (CD21+CD27+) cell subsets and suggest that SIV infection may induce early defective responses in specific B cell subsets in specific tissues.

2. MATERIALS AND METHODS

2.1. Animals, virus, BrdU, and tissue sampling

Twenty female and 2 male Indian RMs (*Macaca mulatta*) between 3–16 years of age, which were initially negative for HIV-2, SIV, type D retrovirus and STLV-1 infection were examined in this study (Table 1). All macaques were given the nucleotide analog BrdU (60mg/kg in sterile saline, Sigma) intraperitoneally 24 hrs prior to euthanasia and tissue collection [17]. Ten female and 2 male RMs were infected either through intravenous, intravaginal or intrarectal route with 10–1000 $TCID_{50}$ SIV_{MAC251}. EDTA anti-coagulated blood, axillary lymph node (ALN), tonsil, spleen, and intestines (jejunum) were collected at necropsy for functional and/or phenotyping experiments. All RMs were housed at the Tulane National Primate Research Center in accordance with the regulations of the American Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and all experiments were reviewed and approved by the Tulane Institutional Animal Care and Use Committee.

2.2. Lymphocyte isolation from tissues

Lymphocytes from the PB, intestine, ALN, tonsil, and spleen were isolated as previously described [17–22]. All cells were washed twice and resuspended in complete RPMI-5 medium containing 5% fetal calf serum (FCS) before staining. All lymphocytes were >90% viable by trypan blue dye exclusion method.

2.3. Immunofluorescent staining and flow cytometric analysis

For flow cytometry staining, cells were adjusted to 10^7 cells/ml and 100ul aliquots or 100ul of whole blood samples were incubated with appropriately diluted concentrations of antibodies for 30 min at 4°C. Whole blood, and spleen samples were then lysed and washed using a whole blood lysis protocol as previously described [19, 23, 24]. BrdU staining was peformed as reported earlier [17, 18]. Cells were kept protected from light at 4°C and acquisition was performed within 24 hrs of staining. Lymphocytes from ALN, jejunum lamina propria lymphocytes (LPL), and tonsil were stained and processed similar to blood tissues with the omission of the whole blood lysing technique [24]. Polychromatic (6–9 parameter) flow cytometric acquisition was performed on a Becton Dickinson LSRII instrument with three lasers (488nm blue laser, 633nm red laser and 407 violet laser) using FITC, PE, PE-Texas red, PE-Cy5, APC, Alexa 700, APC-Cy7, Pacific Blue, and Qdot655 as fluorochrome directly conjugated to antibodies. Single-stained controls for each

fluorochrome were used for compensation settings. Monoclonal antibodies CD3 (SP32-2), CD20 (L27), CD21 (B-ly4), CD27 (M-T271), and BrdU FITC (3D4) were obtained from BD Biosciences [17]. CD8 (MHCD0817) and CD4 Qdot655 (T4/19Thy5D7) were obtained from Invitrogen and the NIH Nonhuman Primate Reagent Resource courtesy of Dr. K. Reimann (Harvard University, Cambridge, MA) respectively. CD27 (0323) was obtained from eBioscience.

At least 30,000 events were collected from each sample by gating on lymphocytes and data were analyzed using FlowJo software (TreeStar Inc.) version 9.1.

2.4. Quantitation of plasma viral RNA

Viral RNA in plasma was quantified by bDNA signal amplification assay (Siemens Diagnostics, CA) [25]. The lower limit of detection was 125 SIV RNA copies/ml of plasma.

2.5. Statistics

Results of experimental groups were compared using either a two-tailed Student's paired t-test or nonparametric Mann-Whitney t test using Prism software (GraphPad software, SanDiego, CA). P values <0.05 were considered significant.

RESULTS AND DISCUSSION

Since we previously showed more DP CD21+CD27+ B cells had the capacity to produce antibodies following polyclonal stimulation (and without T cell help), and higher rates of proliferation than their SP counterparts [17], here we compared differences in proliferation rates in both T and B cell subsets from normal and SIV_{MAC251} infected RMs. Ten normal and 12 SIV_{MAC251} infected RMs (Table 1) were inoculated with BrdU 24 hrs prior to sampling to detect cells in S-phase (DNA synthesis) of division. Of the 12 SIV infected RMs examined, five were in acute infection (8–13 days pi) and seven were in the chronic phase of infection (143–313 days pi).

Plasma viral load and CD4+ T cell percentages from both PB and jejunum LPL tissues were quantified in all 10 normal and 12 SIV_{MAC251} infected RMs. Overall in both the acute and chronically infected RMs there was high plasma viremia proving that all the macaques were infected (Fig. S1). There was no significant difference in CD20+ B cell percentages between normal and acutely infected RMs in all lymphoid and peripheral tissues examined (Fig. S1). However, a significant increase in CD20+ B cells was detected in tonsillar lymphoid tissues compared to PB ($P<0.05$) (Fig. S1). CD20+ B cell percentages remained high in ALN and splenic lymphoid tissues in chronically infected RMs, but data were not significantly different from uninfected RMs.

CD21 surface expression as well as double positive CD21+CD27+ B cell subsets differs between PB and lymphoid B cell population (Fig. 1). By modeling the rate of BrdU uptake in vivo in normal and SIV infected adult RMs, we were able to compare differences in lymphocyte proliferation rates among B-cell subsets and T cells. In normal RMs, there was a higher proliferation in DP CD21+CD27+ B cells in all-lymphoid tissues (Fig. 2A). Significantly higher rates of DP CD21+CD27+ B cell proliferation were observed in tonsil than compared with any other tissue examined. These high rates of DP CD21+CD27+ B cell proliferation may help to maintain the pool of functional B cell populations in normal RMs. However, 8–13 days after SIV infection, there was a significant reduction in tonsillar DP memory B cell proliferation compared to other tissues, suggesting a rapid and severe localized defect occurs in tonsillar B cells in early infection (Fig. 2A; $p<0.01$). Decreased tonsillar B cell proliferation also persisted in chronically infected RMs compared to normal RMs (Fig. 2A; $P<0.05$), but there was a concurrent increased T cell proliferation in all other

tissues examined. Jejunum LPL B cell subsets (DP CD21+CD27+ and SP CD21+CD27−) in chronically infected RMs showed increased proliferation compared to normal macaques, whereas the rate of proliferation of SP CD21−CD27+ and DN CD21−CD27− cells was significantly higher compared to normal RMs (Fig. 2A, $P<0.01$). Combined, these data indicate that in all tissues, DP CD21+CD27+ B cells have much higher rates of proliferation than SP CD21−CD27+ B cells. In addition, DP CD21+CD27+ B cell proliferation increases predominantly due to expansion of preexisting memory cells in most tissues (ALN and Spleen) except tonsil, where the proliferation markedly decreases within days of infection. Progressive depletion of proliferating (Ki67+) B cells has also been reported in lymph node germinal centers at as early as 20 days after SIV_{MAC239}, however, the assay was performed by immunohistochemistry staining [11]. In-contrast, there was a significant increase in T cell proliferation in chronically infected RMs compared to acutely infected and normal RMs in all tissues examined (Fig. 2B, $p<0.01$). The increased T cell proliferation during chronic infection in tissues is consistent with generalized immune activation [26, 27].

Decreased proliferation of memory (CD21+CD27+) B cells in tonsil is suggestive of an early B cell defect localized in this tissue which might be a key feature in the failure of functional B cell responses in SIV infection, as these DP CD21+CD27+ B cells are capable of generating more antibodies by polyclonal activation and in the absence of T cell help [28]. These results demonstrate important information about the biology of tissue specific B lymphocyte proliferation in RMs, and suggest the intriguing possibility that tissue specific B cell dysfunction may play an important role in SIV pathogenesis. In summary, these data demonstrate differences in nonhuman primate B cell subsets proliferation in selective tissues, and suggest that SIV infection may induce early selective defective responses in specific tissues compared to T cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- DP CD21+CD27+ B cells were able to become activated and proliferate at higher rates.
- Increased proliferation of tonsillar memory B cells were identified.
- Decreased proliferation of tonsillar memory B cells during acute SIV infection.

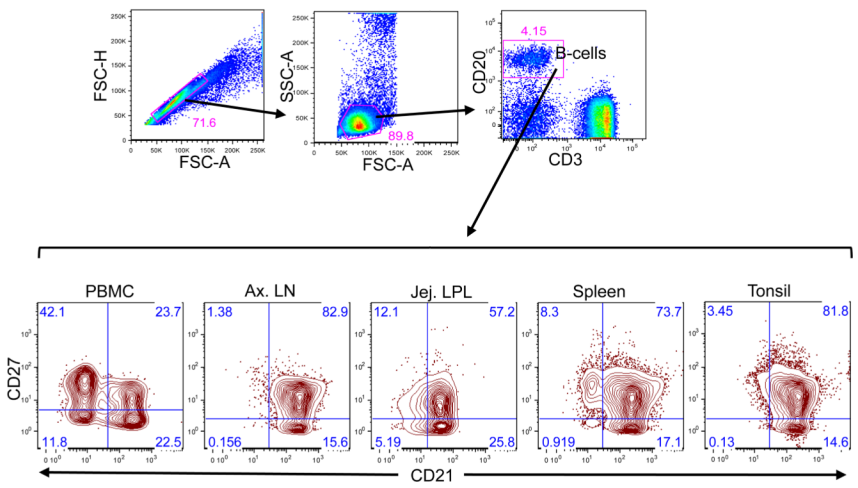

Figure 1.

A representative dot and contour plots showing distribution of CD21 and CD27 phenotype on CD20+ B cells from a normal uninfected healthy rhesus macaque (GN74). Singlets were gated first followed by lymphocytes and finally gating was performed in CD20+ B lymphocytes. All CD20+ B lymphocytes were further analyzed based on their CD21 and CD27 surface molecule expression. Each quadrant shows percentages of specific B cell subset populations.

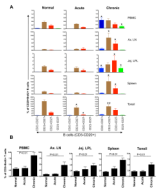

Figure 2.

Bar graphs showing the mean BrdU+ proliferative responses in different CD20+ B cell subsets (**A**) and CD3+ T cells (**B**) from different tissues of normal and SIV infected macaques in acute and chronic infection. BrdU was injected intraperitoneally and tissues were collected 24 hrs after inoculation. (**A**) Higher proliferation of CD21+CD27+ B cell subsets compared to other B cells subsets were observed in all healthy normal rhesus macaques. Interestingly tonsillar CD21+CD27+ B cell proliferation decrease in both acute and chronic SIV infection compared to normal healthy macaques. ℙ($p<0.01$) and ℙ ℙ ($p<0.05$) indicate significant differences between the specified cell subsets and the same subset from normal groups. (B) A significant increase in T cell proliferation in chronically infected RMs compared to acutely infected and normal RMs in all tissues was detected.

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Table 1

List of adult Indian Rhesus Macaques examined

Category	Animal Number	Sex [a]	Virus	Days of infection	Dosage	Route [b]
Normal	GN70	F	Nil		-	-
	GN66	F	Nil		-	-
	GN58	F	Nil		-	-
	BB01	F	Nil		-	-
	N483	F	Nil		-	-
	GN74	F	Nil		-	-
	CC10	F	Nil		-	-
	AG71	F	Nil		-	-
	HI55	F	Nil		-	-
	DJ78	F	Nil		-	-
Acute Infection	T108	F	SIV_{MAC251}	8	$500TCID_{50}$	IV
	M992	F	SIV_{MAC251}	13	$500TCID_{50}$	IV
	AV91	M	SIV_{MAC251}	10	$500TCID_{50}$	IV
	BA57	F	SIV_{MAC251}	8	$500TCID_{50}$	IV
	HI52	F	SIV_{MAC251}	8	$500TCID_{50}$	IV
Chronic Infection	DV42	F	SIV_{MAC251}	227	$500TCID_{50}$	IVAG
	HG56	F	SIV_{MAC251}	154	$300TCID_{50}$	IV
	HG49	F	SIV_{MAC251}	147	$300TCID_{50}$	IVAG
	HG58	F	SIV_{MAC251}	288	$300TCID_{50}$	IVAG
	FE53	M	SIV_{MAC251}	143	$10TCID_{50}$	IR
	EJ26	F	SIV_{MAC251}	308	$100TCID_{50}$	IV
	DR59	F	SIV_{MAC251}	313	$1000TCID_{50}$	IVAG

[a] F and M denote female and male respectively.

[b] IV, IVAG and IR denote intravenous, intravaginal and intrarectal route respectively.

All RMs received BrdU intraperitoneally 24h before sampling.