Published in final edited form as:

J Med Primatol. 2014 October ; 43(5): 374–377. doi:10.1111/jmp.12109.

PAS-Positive Extracellular Deposits within Germinal Centers of Hyperplastic Follicles during SIV infection in a Rhesus Macaque

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Abstract

Background—Lymphoid tissue remodeling is characteristic of chronic SIV infection.

Methods—A rhesus macaque infected with SIVmac239 was necropsied and its lymphoid tissues subjected to histopathology characterization.

Results—Germinal centers in spleen and lymph nodes contained PAS-positive, non-amyloid extracellular deposits, decreased T follicular helper cells, and normal density of Ki67⁺ B cells.

Conclusions—A possible mechanism for PAS-positive deposits includes exaggerated involution of SIV-induced follicular hyperplasia secondary to virus-associated immune reaction.

Keywords

non-amyloid; *Macaca mulatta*; spleen; lymph node; T follicular helper cells

Introduction

Lymphoid tissue remodeling represents a hallmark of chronic SIV and HIV infection resulting from the continuous inflammatory processes and immune activation. Thus, the lymph node architecture of SIV infected monkeys present with highly organized lymphoid follicles with prominent germinal centers (GCs). Here we report the features of non-amyloid non-fibrillary PAS-positive deposits within GCs of hyperplastic follicles and GC-associated immune responses caused by SIV. To our knowledge, this is the first report of PAS-positive deposition within GCs showing the defect of T_{FH} cells identified in rhesus macaques.

Materials and Methods

The subject (RAR7) was a 6 year old intact male rhesus macaque born at the Yerkes National Primate Research Center (YNPRC) and assigned to a research protocol approved by the Emory University Institutional Animal Care and Use Committee. YNPRC is fully accredited by AAALAC. The macaque was fed a commercial primate chow (Monkey Diet 5037 Lab

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Diet, PMI Nutritional International, St. Louis, MO) with daily supplementation of a variety of fresh fruits and vegetables.

The animal was assigned to a research protocol that studied immune restoration via transfusions of autologous in vitro expanded CD4⁺ T cells to SIV-infected macaques [9,10,13]. The subject was intravenously infected with SIVmac239. A complete necropsy was performed at 54 weeks post infection. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 6 µm. Sections of spleen, axillary lymph nodes, and ileo-ceco-colon tissues were stained with hematoxylin and eosin (HE), Periodic acid Stiff hematoxylin (PASH), periodic acid-Schiff (PAS) with and without diastase digestion, Congo red, Sulfated Alcian Blue (SAB), phosphotungstic acid hematoxylin (PTAH), and Masson's Trichrome stains to elucidate the observed amorphous eosinophilic material noted in the HE-stain tissue sections.

Immunohistochemical analysis was performed on paraffin sections of formalin-fixed tissue. The antibodies used were anti-kappa light chain and lambda light chains. To examine GC-associated immune responses in follicles with PAS-positive deposits, the axillary lymph node, spleen, and colon were stained with goat anti-human PD-1 (R&D system, Minneapolis, MN), anti-human Ki67 (Vector, Burlingame, CA) and rabbit anti-human CD20 (Thermo scientific, Rockford, IL) antibodies during the first incubation. Alexa fluor 488/Cy3/Cy5 conjugated donkey anti-mouse/rabbit/goat antibodies (Jackson ImmunoResearch, West Grove, PA) were used during the second incubation.

Electron microscopy evaluation was performed on formalin-fixed spleen.

Results

Three days prior to necropsy, this animal had an acute onset of watery diarrhea. Decreased activity, anorexia and weight loss were observed. Clinical pathology results showed moderate azotemia (BUN 143 mg/dL normal reference range 8 – 30 mg/dL; creatinine 7.7 mg/dL normal reference range 0.8 – 2.3 mg/dL) and neutrophilia. Findings at necropsy included slightly enlarged spleen, moderately enlarged axillary and inguinal lymph nodes, and fluid filled intestines. Histopathology included interstitial pneumonia due to opportunistic infection with *Pneumocystis carinii*. The plasma viral load was 1.97×10^5 mRNA copies per ml plasma.

Microscopic examination of the spleen revealed abundant extracellular amorphous deposits that were confined to the central areas of the GCs of the splenic white pulp. The deposits were eosinophilic with HE staining, PAS-positive, and negative for amyloid with Congo red and SAB histochemical stains, and negative for collagen with Masson's Trichrome stain (Fig. 1). Similar deposits were present in the follicles of axillary lymph node and colonic GALT. Staining for kappa and lambda light chains was negative. Special stains failed to reveal the exact nature of the deposits.

The axillary lymph node, spleen, and colonic lymphoid tissue showed that the PAS-positive deposits were surrounded by Ki67⁺ B cells, but the density of PD-1^{hi} follicular T helper

cells were markedly reduced as compared to typical hyperplastic follicles in lymphoid tissues (Fig 2) [6].

Ultrastructural observations revealed the extracellular deposits had a non-fibrillar, granular electron-dense appearance (Data not shown).

Discussion

Here, we report the accumulation of PAS-positive, non-amyloid deposits in GCs of spleen, lymph nodes, and colonic GALT of a rhesus macaque infected during chronic SIV infection.

Extracellular PAS-positive deposits have been observed in reactive lymphoid follicles of spleens at autopsy from HIV-infected patients [14] but have not been described in lymphoid tissues of SIV infected monkeys.

Non-amyloid deposits have been reported in lymphoproliferative B-cell conditions such as reactive follicular hyperplasia, follicular lymphoma, plasma cell dyscrasias, and dysimmune disorders. In patients with light or heavy chain deposition diseases [8,12], and in a patient with rheumatoid arthritis [2], the non-amyloid deposits may represent abnormal products of the immune reaction such as monoclonal immunoglobulin [1,3,5,7,11]. These deposits must be differentiated from amyloid deposits found in follicular lymphoma, and from stromal reactions variously described as hyalinosis, fibrosis, or sclerosis [5].

Only amyloid deposits stain for Congo red, a tinctorial property related to the β -pleated sheet organization of amyloid fibrils common to all forms of amyloid. Electron microscopy is helpful in distinguishing between both these lesions. Amyloid deposits are characteristically fibrillar, whereas the PAS-positive deposits are ultrastructurally non-organized non-fibrillar, granular appearance [3].

Interestingly, this animal showed marked decreases of GC PD-1^{hi} cells, even though a normal accumulation of Ki67⁺CD20⁺ cells surrounding amorphous deposits were present in the center of hyperplastic follicles (Figure 2). Indeed, our data showed that Ki67⁺CD20⁺ cells and PD-1^{hi} cells were dramatically increased in hyperplastic follicles of lymph nodes during chronic SIV infection [6]. PD-1^{hi} cells within GCs were positive for CD3 and CD4, not CD8 [6]. Moreover, these PD-1^{hi} CD4 T cells in lymphoid tissues relatively express high level of ICOS, CD200 and CXCR5 [4,6], suggesting they are phenotypically GC-associated follicular CD4⁺ helper T (T_{FH}) cells that deliver help for B cell maturation. There is no direct pathologic connection between a precipitate of non-amyloid substance and a defect in GC T_{FH} cells during SIV infection. Moreover, the pathologic significance of this substance within GCs has not been reported in human and any other animal models. It is possible that the abnormality of B cell immunity might be due to the reduced T_{FH} cells, leading to the accumulation of non-amyloid substances from B cells within GCs [5]. Since this animal was chronically infected with SIV, the chronic viral replication might have contributed to this histologic presentation. In general, SIV slow progressors display hyperplastic follicles with an abundance of T_{FH} cells and proliferating B cells within GCs [6]. Rapid SIV progressors which develop AIDS before 6 months, show involution of GCs at necropsy time points. Therefore, the interesting correlations between protein deposition

and GC-related immune responses in an immunosuppressive situation should be addressed in future study.

Acknowledgments

This work was supported by grants NIH 1RO1 HL075833 and Yerkes base grant P51OD11132. The authors are indebted to the staff and veterinarians of the Yerkes National Primate Research Center for their excellent care of the animals. We thank the Emory CFAR Virology Core for viral RNA load determinations.

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Figure 1.

Spleen of rhesus macaque 54 weeks post infection with SIVmac239: Amorphous extracellular deposits within white pulp are strongly PAS-positive; appear eosinophilic in HE section; are negative for AA-amyloid with Congo red and sulfated Alcian blue staining; and negative for collagen with Masson's Trichrome staining.

Figure 2.

Germinal Center-associated immune cells (TFH cells and GC B cells) in axillary lymph node, spleen and colon of rhesus macaque 54 weeks post infection with SIVmac239: the PAS-positive deposits are surrounded by Ki67⁺ B cells, but the density of PD-1^{hi} follicular T helper cells is reduced as compared to typical hyperplastic follicles in lymphoid tissues.