Published in final edited form as:

J Med Primatol. 2011 June ; 40(3): 200–204. doi:10.1111/j.1600-0684.2011.00473.x.

Hypergammaglobulinemia in an SIV-Infected Rhesus Macaque with a B cell neoplasm with plasma cell differentiation

Joshua A. Kramer, Brigitte E. Sanders-Beer¹, Yvette Edghill Spano², Lowrey Rhodes Jr., and Keith G. Mansfield

Harvard Medical School, New England Primate Research Center, Southborough, MA (JAK, KGM)
Southern Research Institute, Frederick, MD (BSB, YES, LR)

Abstract

An SIV-infected rhesus macaque presented with anemia, hypercalcemia, and hyperglobulinemia. Neoplastic round cells with plasma cell morphology infiltrated multiple organs and stained immunohistochemically positive for CD45, MUM1/IRF4, CD138, VS38C, and Kappa light chain and variably positive for CD20 and CD79a; consistent with a B-cell neoplasm with plasma cell differentiation.

Keywords

Rhesus macaque; SIV; hypergammaglobulinemia; lymphoproliferative disorder; plasma cell

Case Report

Though relatively frequent in HIV-infected people [5,7,9], monoclonal gammopathy has never been reported in an SIV-infected macaque. Herein, we describe the clinicopathologic, histologic, and immunohistochemical findings from an SIVmac251-infected adult female Chinese-origin rhesus macaque participating in a topical microbicide study that presented with abnormal hyperglobulinemia and was subsequently diagnosed with B-cell neoplasm with significant plasma cell differentiation. The animal was enrolled on the same study with five other animals, none of which showed the same signs.

The animal had tested seronegative for Simian T-Lymphotropic Virus, Simian Retrovirus Type D, and Herpes B virus before it was treated with 30mg Depo-Provera[®] intramuscularly and challenged 30 days later intravaginally with 237 TCID₅₀ SIVmac251/CEMx174. The animal developed recurrent and treatment-resistant diarrhea, anorexia, weight loss, and ataxia, and was euthanized 20 months after infection in response to its deteriorating clinical condition.

At the time of necropsy, the animal had high plasma viral loads (2.6×10^5 RNA copies/mL), but the peripheral CD4 count was preserved (1,701 cells/ μ L). The serum chemistry profile showed hyperproteinemia (12.2 g/dL), hyperglobulinemia (10.2 g/dL), hypoalbuminemia (2.0 g/dL), azotemia (BUN 75 mg/dL), and hypercalcemia (13.0 mg/dL) which became more apparent when corrected for hypoalbuminemia (14.9 mg/dL). The animal was anemic

Corresponding author: Keith Mansfield D.V.M. Harvard Medical School New England Primate Research Center 1 Pine Hill Drive Southborough, MA 01772 Tel: 508-624-8183 Fax: 508-624-8135 keith_mansfield@hms.harvard.edu.

¹Current address: BIOQUAL, Inc., Rockville, MD 20850

²Current address: National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

(hematocrit 30.3% and hemoglobin 8.9 g/dL) and thrombocytopenic ($96 \times 10^3/\text{mm}^3$), with leukocytosis ($20.26 \times 10^3/\text{mm}^3$).

There were marked increases in the number of circulating CD20-positive B-lymphocytes as determined by flow cytometry (Figure 1a). Serum electrophoresis showed an increase in the gamma globulin fraction over time. Initially, the increase in gamma globulin appeared polyclonal in nature (Figure 1c). However, by seventeen months post infection, there was a clear monoclonal spike (Figure 1d). The percent gamma globulin fraction rose from 10.3% to 43.6 while the absolute level rose to 5.3 g/dL. Other animals in the same experimental group showed less intense, polyclonal increases in the gamma globulin fraction without a monoclonal spike (Table 1).

At necropsy, there were white nodules in the spleen, liver, heart, and kidneys corresponding to dense infiltrates of neoplastic round cells. Similar cells infiltrated several lymph nodes and the bone marrow (Figure 2). The infiltrates were composed of mature plasma cells admixed with large pleomorphic cells containing abundant eosinophilic cytoplasm, often forming nodules in tissues. In the heart, approximately 30% of the examined section was characterized by cellular infiltrates expanding between myofibers and was associated with myocardial degeneration and necrosis.

In addition, there was granulomatous colitis secondary to *Mycobacterium avium* complex infection, progressive multifocal leukoencephalomalacia secondary to Simian Virus 40 infection, SIV giant cell pneumonia, moderate to severe glomerulosclerosis, and renal tubular proteinosis with protein cast formation.

In light of the serum electrophoresis data and to further characterize the neoplastic cellular proliferations, a series of immunohistochemical stains was performed using previously described methods [3,23,24]. Results from immunohistochemical staining are summarized in Table 2.

In man, the differentiation of various disorders causing a monoclonal gammopathy is important from both a diagnostic and prognostic perspective. Unfortunately, the clinical workup before necropsy in this case was somewhat limited and precludes us from reaching a final diagnosis. Differential diagnoses include multiple myeloma, plasma cell leukemia, Waldenstrom's macroglobulinemia, plasmablastic lymphoma, and B-cell lymphoma with plasma cell differentiation.

Immunohistochemical staining confirmed the plasma cell origin of a large percentage of the neoplastic cells. Both VS38C and CD138 are plasma cell specific and stained exclusively plasma cells in rhesus control tissues [15,16,20]. Interestingly, CD138 is a surface oriented marker in man but in both this case and in rhesus control tissues, staining was cytoplasmic (figures 2B and 2J). Positive staining for MUM1/IRF4 and CD79a, although not specific for plasma cells, is also reported in the majority of canine and human tumors with plasma cell differentiation [8,18,19].

The increased CD20 staining and lack of CD56 expression is most consistent with a B-cell lymphoma or plasmablastic lymphoma. These tumors can have significant plasma cell differentiation and, rarely, hypergammaglobulinemia [4,13]. However, CD20 is expressed in up to 17% of human multiple myeloma cases [6,14,18,19] and while CD56 is expressed in most myelomas [1], it is often absent in advanced cases of multiple myeloma and plasma cell leukemia [12,19]. Waldenstrom's macroglobulinemia is associated with monoclonal gammopathies and can have a VS38C+/CD138+ component. However, it is associated with IgM production and generally follows a more insidious course [22].

We favor a diagnosis of either multiple myeloma with extramedullary spread or plasma cell leukemia. The diagnostic criteria presented by the International Myeloma Working Group (IMWG) to differentiate multiple myeloma from other causes of monoclonal gammopathy includes three criteria: 1) serum or urine M-protein ≥ 3.0 g/dL, 2) bone marrow plasma cells $\geq 10\%$, and 3) end organ or tissue impairment (hypercalcemia, anemia, etc) [11].

The histologic and immunohistochemical findings of this case, together with monoclonal gammopathy, gamma fraction > 3.0 g/dL, hypercalcemia, anemia, and bone marrow involvement are supportive of a diagnosis of multiple myeloma. Unfortunately, due to this animal's comorbidities, we cannot be certain that the neoplasm caused the anemia and the hypercalcemia, precluding us from reaching a definitive diagnosis. Additionally, diagnostics recommended by the IMWG including radiographs and serum/urine immunofixation were not performed prior to necropsy. Evaluation of a blood smear prior to necropsy would have enabled us to determine if the animal had progressed to plasma cell leukemia [12].

Regardless of the final diagnosis, reports of tumors with plasma cell differentiation in nonhuman primates are exceedingly rare [2,10,17,21]. Additionally, the cardiac involvement in this case is unique when compared to human tumors, and is likely indicative of the aggressive nature of this tumor. We hypothesize that there is a similar pathogenesis in this case to that proposed for HIV-infected people, whereby continuous antigenic stimulation of B-cells by SIV was the initiating event in neoplastic transformation.

We have described the histological and immunohistochemical findings of an aggressive B-cell neoplasm with significant plasma cell differentiation presenting as a monoclonal gammopathy in an SIV-infected rhesus macaque. Lymphoproliferations with plasma cell differentiation and plasma cell disorders should be considered as differential diagnoses for hypergammaglobulinemia in rhesus macaques. Additional investigations to determine the incidence of monoclonal gammopathy in SIV-infected macaques are warranted.

Acknowledgments

The authors would like to thank Audra Hachey and Christine Pearson for help with immunohistochemical staining, Kristen Toohey for help with graphics, and Dr. Ron Desrosiers for providing the SIVmac251/CEMx174 challenge stock.

Funding source: This work was supported in part by NIH/ NCRR Grant P51 RR00168-48 and NIH contract N01-AI-15451.

References

1. Bataille R, Jigo G, Robillard N, Barille-Nion S, Harousseau JL, Moreau P, Amiot M, Pellat-Deceunynck C. The phenotype of normal, reactive and malignant plasma cells. Identification of "many and multiple myelomas" and of new targets for myeloma therapy. *Haematologica*. 2006; 91:1234–1240. [PubMed: 16956823]
2. Broerse JJ, Bartstra RW, van Bekkum DW, van der Hage MH, Zurcher C, van Zwieten MJ, Hollander CF. The carcinogenic risk of high dose total body irradiation in non-human primates. *Radiother Oncol*. 2000; 54:247–253. [PubMed: 10738083]
3. Carville A, Mansfield KG. Comparative pathobiology of macaque lymphocryptoviruses. *Comp Med*. 2008; 58:57–67. [PubMed: 19793458]
4. Chetty R, Hlatwayo N, Muc R, Sabaratnam R, Gatter K. Plasmablastic lymphoma in HIV+ patients: an expanding spectrum. *Histopathology*. 2003; 42:605–609. [PubMed: 12786898]
5. Cheung MC, Pantanowitz L, Dezube BJ. AIDS-related malignancies: emerging challenges in the era of highly active antiretroviral therapy. *Oncologist*. 2005; 10:412–426. [PubMed: 15967835]
6. Deshmukh M, Elderfield K, Rahemtulla A, Naresh KN. Immunophenotype of neoplastic plasma cells in AL amyloidosis. *J Clin Pathol*. 2009; 62:724–730. [PubMed: 19638544]

7. Dezube BJ, Aboulafia DM, Pantanowitz L. Plasma cell disorders in HIV-infected patients: from benign gammopathy to multiple myeloma. *AIDS Read*. 2004; 14:372–374. 377–379. [PubMed: 15282866]
8. Fernandez NJ, West KH, Jackson ML, Kidney BA. Immunohistochemical and histochemical stains for differentiating canine cutaneous round cell tumors. *Vet Pathol*. 2005; 42:437–445. [PubMed: 16006603]
9. Fiorino AS, Atac B. Paraproteinemia, plasmacytoma, myeloma and HIV infection. *Leukemia*. 1997; 11:2150–2156. [PubMed: 9447834]
10. Hollander CF, Zurcher C, Broerse JJ. Tumorigenesis in high-dose total body irradiated rhesus monkeys--a life span study. *Toxicol Pathol*. 2003; 31:209–213. [PubMed: 12696581]
11. IMWG. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *Br J Haematol*. 2003; 121:749–757. [PubMed: 12780789]
12. Jimenez-Zepeda VH, Dominguez-Martinez VJ. Plasma cell leukemia: a highly aggressive monoclonal gammopathy with a very poor prognosis. *Int J Hematol*. 2009; 89:259–268. [PubMed: 19326058]
13. Kyle RA, Rajkumar SV. Monoclonal gammopathy of undetermined significance. *Clin Lymphoma Myeloma*. 2005; 6:102–114. [PubMed: 16231848]
14. Mateo G, Castellanos M, Rasillo A, Gutierrez NC, Montalban MA, Martin ML, Hernandez JM, Lopez-Berges MC, Montejano L, Blade J, Mateos MV, Sureda A, de la Rubia J, Diaz-Mediavilla J, Pandiella A, Lahuerta JJ, Orfao A, San Miguel JF. Genetic abnormalities and patterns of antigenic expression in multiple myeloma. *Clin Cancer Res*. 2005; 11:3661–3667. [PubMed: 15897562]
15. Ng AP, Wei A, Bhurani D, Chapple P, Feleppa F, Juneja S. The sensitivity of CD138 immunostaining of bone marrow trephine specimens for quantifying marrow involvement in MGUS and myeloma, including samples with a low percentage of plasma cells. *Haematologica*. 2006; 91:972–975. [PubMed: 16818287]
16. O'Connell FP, Pinkus JL, Pinkus GS. CD138 (syndecan-1), a plasma cell marker immunohistochemical profile in hematopoietic and nonhematopoietic neoplasms. *Am J Clin Pathol*. 2004; 121:254–263. [PubMed: 14983940]
17. Radl J, Liu M, Hoogeveen CM, van den Berg P, Minkman-Brondijk RJ, Broerse JJ, Zurcher C, van Zwieten MJ. Monoclonal gammopathies in long-term surviving rhesus monkeys after lethal irradiation and bone marrow transplantation. *Clin Immunol Immunopathol*. 1991; 60:305–309. [PubMed: 2070572]
18. Ramos-Vara JA, Miller MA, Valli VE. Immunohistochemical detection of multiple myeloma 1/interferon regulatory factor 4 (MUM1/IRF-4) in canine plasmacytoma: comparison with CD79a and CD20. *Vet Pathol*. 2007; 44:875–884. [PubMed: 18039900]
19. San Miguel JF, Gutierrez NC, Mateo G, Orfao A. Conventional diagnostics in multiple myeloma. *Eur J Cancer*. 2006; 42:1510–1519. [PubMed: 16762540]
20. Turley H, Jones M, Erber W, Mayne K, de Waele M, Gatter K. VS38: a new monoclonal antibody for detecting plasma cell differentiation in routine sections. *J Clin Pathol*. 1994; 47:418–422. [PubMed: 7517959]
21. Uno H, Warner TF. Plasmacytomas in rhesus monkeys. *Arch Pathol Lab Med*. 1982; 106:278–281. [PubMed: 6896433]
22. Vijay A, Gertz MA. Waldenstrom macroglobulinemia. *Blood*. 2007; 109:5096–5103. [PubMed: 17303694]
23. Yearley JH, Pearson C, Carville A, Shannon RP, Mansfield KG. SIV-associated myocarditis: viral and cellular correlates of inflammation severity. *AIDS Res Hum Retroviruses*. 2006; 22:529–540. [PubMed: 16796528]
24. Yearley JH, Pearson C, Shannon RP, Mansfield KG. Phenotypic variation in myocardial macrophage populations suggests a role for macrophage activation in SIV-associated cardiac disease. *AIDS Res Hum Retroviruses*. 2007; 23:515–524. [PubMed: 17506608]

Figure 1.

Graph of CD20 B cells/ μ L and serum electrophoresis results. **Fig 1a.** CD20 B cells/ μ L as a function of time post SIV infection. **Fig 1b.** Serum electrophoresis at baseline (point “b” on B cell graph). Peaks correspond to albumin, alpha 1, alpha 2, beta and gamma globulin fractions. **Fig 1c.** Serum electrophoresis at peak B cell levels (58 weeks post SIV infection, point “c” on B cell graph) shows a broad peak in the gamma globulin fraction. **Fig 1d.** Serum electrophoresis after B cell levels returned to baseline (79 weeks post inoculation, point “d” on B cell graph) showing development of a monoclonal peak in the gamma globulin fraction.

Figure 2.

Microscopic and immunohistochemical findings. **Fig 2A.** Bone marrow; rhesus macaque. Infiltrate of neoplastic plasma cells. HE. **Fig 2B.** Bone marrow; rhesus macaque. Numerous cells throughout the bone marrow stain positive for the plasma cell marker CD138. Anti-CD138 immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2C.** Bone marrow; rhesus macaque. Numerous cells throughout the bone marrow stain positive for the plasma cell marker VS38C. Anti-VS38C immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2D.** Bone marrow; rhesus macaque. CD20 staining is largely absent from the bone marrow with only scattered CD20+ cells. Anti-CD20 immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2E.** Bone marrow; rhesus macaque. Most neoplastic cells stain positive for MUM1/IRF-4. Anti-MUM1 immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2F.** Bone marrow; rhesus macaque. A large number of neoplastic cells stain strongly positive for kappa light chain. Anti-Kappa light chain immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2G.** Bone marrow; rhesus macaque. The bone marrow is nearly completely negative for lambda light chain. Anti-Lambda light chain immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2H.** Bone marrow; rhesus macaque. The bone marrow is nearly completely negative for IgM. Anti-IgM immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2I.** Spleen; rhesus macaque. Marked infiltrate of neoplastic round cells with plasma cell morphology. HE. **Fig 2J.** Spleen; rhesus macaque. Numerous CD138+ plasma cells throughout the neoplastic infiltrate. Anti-CD138 immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2K.** Spleen; rhesus macaque. Numerous VS38C+ plasma cells throughout the neoplastic infiltrate. Anti-VS38C immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2L.** Spleen; rhesus macaque. Neoplastic cells are variably CD20+ with some areas (upper part of image) being strongly positive while others (lower half of image) have only scattered CD20+ cells. Anti-CD20 immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2M.** Spleen;

rhesus macaque. Most neoplastic cells stain positive for MUM1/IRF-4. Anti-MUM1 immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2N.** Spleen; rhesus macaque. Many neoplastic cells are strongly positive for kappa light chain. Anti-Kappa light chain immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2O.** Spleen; rhesus macaque. Neoplastic cells are diffusely negative for lambda light chain. Lambda light chain immunohistochemistry, DAB chromogen, hematoxylin counterstain. **Fig 2P.** Spleen; rhesus macaque. Neoplastic cells are negative for IgM. A few, scattered, individual cells stained IgM+ but this is insignificant overall. Anti-IgM immunohistochemistry, DAB chromogen, hematoxylin counterstain.

Changes in serum electrophoresis results from the presented case and unaffected animals in the same experimental group at baseline numbers of B cells, peak of B cells, and after return to baseline numbers of B cells.

Table 1

	Baseline Unaffected ¹	Baseline Affected	Peak B cells Unaffected ^{1,2}	Peak B Cells Affected	Return to Baseline Unaffected ^{1,3}	Return to Baseline Affected
albumin	70.76 (2.01)	56.0	61.36 (3.41)	41.8	57.86 (2.58)	29.5
alpha1	2.76 (0.18)	5.1	3.24 (0.53)	2.8	4.02 (0.91)	3.9
alpha2	12.40 (1.35)	19.7	13.14 (1.38)	17.8	14.98 (3.41)	18.7
Beta	6.64 (2.38)	8.7	7.34 (1.29)	6.5	7.30 (1.22)	4.4
gamma	7.46 (1.94)	10.5	14.94 (3.16)	31.1	15.84 (2.21)	43.6

¹ Values are shown as mean (SD).

² Time of peak B cells varied, but was between 49-58 weeks post-inoculation.

³ Time at return to baseline = 79 weeks

Table 2

Results of Immunohistochemical staining.

Immunostain	Spleen	Liver	Lymph Node	Bone Marrow
CD3	– *	– *	– *	– *
TIA-1	– *	–	– *	–
CD20	+/-	–	+/-	–
CD79a	–	–	faint +	faint +
Ham56	– *	– *	– *	–
CD45	+	+	+	+
MUM1/IRF4	+	+	+	+
κ Light Chain	+	+	+	+
λ Light Chain	–	–	–	–
IgM	–	–	–	–
IgA	–	–	–	–
CD138	+	+	+	+
CD56	–	–	–	–
VS38c	+	+	+	+

* = majority of cells in neoplastic areas negative but scattered + cells throughout interpreted as infiltrating and/or residual cells.