JOURNAL OF VIROLOGY, June 1997, p. 4809–4814 Vol. 71, No. 6
0022-538X/97/$04.00+0

Human Immunodeficiency Virus-Associated Vasculopathy in Transgenic Mice

BRAD T. TINKLE,[1] LIEN NGO,[1] PAUL A. LUCIW,[3] TOM MACIAG,[2] AND GILBERT JAY[1]*

Departments of Virology[1] and Molecular Biology,[2] Jerome H. Holland Laboratory, Rockville, Maryland 20855, and Department of Medical Pathology, University of California, Davis, California 95616[3]

Received 15 October 1996/Accepted 13 March 1997

There is substantial clinical evidence for the development of vascular disorders in human immunodeficiency virus (HIV)-infected individuals, particularly in the form of vasculitis. Transgenic mice carrying a replication-defective HIV-1 provirus with selective deletion of the *gag*, *pol*, and *env* genes developed extensive vasculopathy. Restricted expression of HIV nonstructural genes in smooth muscle cells was accompanied by the migration and proliferation of these cells in blood vessels of all sizes and at different body sites. The frequent infiltration observed in the hypertrophic vessel walls occurred predominantly in the adventitia and was composed of primarily T cells and occasionally plasma cells. The intimal thickening generated significant luminal narrowing in some vessels, and the restricted blood flow led to ischemia in the affected tissues. Interestingly, the endothelium did not appear to support HIV gene expression or be involved in the pathological process. This transgenic model provides an opportunity to dissect the mechanism underlying HIV-associated vasculopathy.

Human immunodeficiency virus type 1 (HIV-1) is capable of infecting multiple cell types either through the cell surface CD4 receptor or through various non-CD4 mechanisms (10, 14, 23, 36). The pathological consequences may depend on the cell type(s) involved. For example, the immune dysfunction observed among HIV-1-infected individuals is a consequence of infection of one or more types of leukocytes (11) whereas infection of renal epithelial cells may result in HIV-associated nephropathy (2).

Several vascular disorders have been documented as a consequence of HIV-1 infection. These include systemic vasculitis (4, 6, 15, 16, 20, 21, 24, 34), formation of "cotton-wool spots" in the eye due to vasculitis-induced ischemic injury (18, 26), and thrombotic thrombocytopenic purpura (7, 8, 19). The vasculitis affects small and medium-sized vessels and is apparently more common among pediatric AIDS patients (6, 20, 21). It is characterized by intimal and medial thickening, resulting from hyperplasia of smooth muscle cells (SMCs). The vascular and perivascular infiltrates are composed of mainly T lymphocytes and mononuclear cells and a few plasma cells. A similar finding has also been made with simian immunodeficiency virus infection of rhesus monkeys, in which approximately 20% of the animals develop arteriopathy (5).

HIV-1-infected patients typically express autoantibodies and circulating immune complexes (32), and the vasculitis observed among infected patients has been attributed to immune complex formation or deposition in a few of the reported cases (15, 18). However, some of the systemic vascular lesions involving SMC proliferation observed associated with HIV-1 infection do not appear to involve an inflammatory response (20, 21). In addition, HIV-1 antigens can be found in vascular and perivascular cells but not in the infiltrate, which is composed primarily of $CD8^+$ T cells (4, 15). Therefore, it remains unclear whether inflammatory cells are necessary to induce the SMC migration and proliferation or are a result of vascular injury.

We have generated transgenic mice carrying a defective HIV-1 provirus (strain SF2) with specific deletion of the *gag*, *pol*, and *env* genes (33) (Fig. 1). Mice from two independent transgenic lines were analyzed for histopathological evidence of vascular lesions. A survey of blood vessels detected in paraffin-embedded sections of the major tissues from each mouse has revealed characteristic vascular changes in over 60% of transgenic animals (11 of 18 mice between 9 and 20 months of age from the low-expressing N4 line and 6 of 10 mice between 27 and 46 days of age from the high-expressing Q5 line), but in none of their nontransgenic littermates. These lesions were noted in vessels of different sizes and were present in various organs, including the brain, heart, kidney, pancreas, mesentery, uterus, and spleen.

Figure 2a shows a renal artery from a healthy, nontransgenic control animal. This vessel exhibits a single layer of luminal vascular endothelium, two to three layers of SMCs in the media, and an outer collagen-enriched adventitia. In contrast, the renal artery of a transgenic mouse shows extensive cellular infiltrates within the vessel wall (Fig. 2b). This artery exhibits an irregular pattern of SMCs that are hyperplastic and that have rounded vesicular nuclei. These lesions are observed at multiple sites within the same animal. The vascular infiltrates in the transgenic mice are localized primarily to the adventitia but can be seen infrequently transcending the medial and intimal layers (Fig. 2c). This infiltrate is composed primarily of lymphoid cells, with scattered plasma cells and sparse monocytes but no granulocytes. The predominant T-cell infiltration is confirmed by the use of an antibody directed against CD3 (data not shown). However, some vessels demonstrate no im-

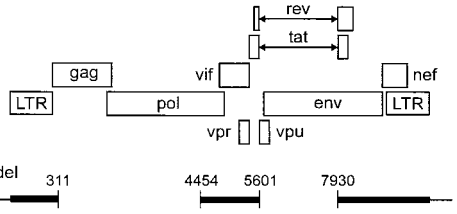

FIG. 1. Schematic of the replication-defective HIV provirus used for producing transgenic mice. The pHIV-del construct contains two deletions, located between nucleotides 311 to 4454 and 5601 to 7930. This modified provirus is shown colinear with the 5′ and 3′ LTRs, as well as the open reading frames.

* Corresponding author.

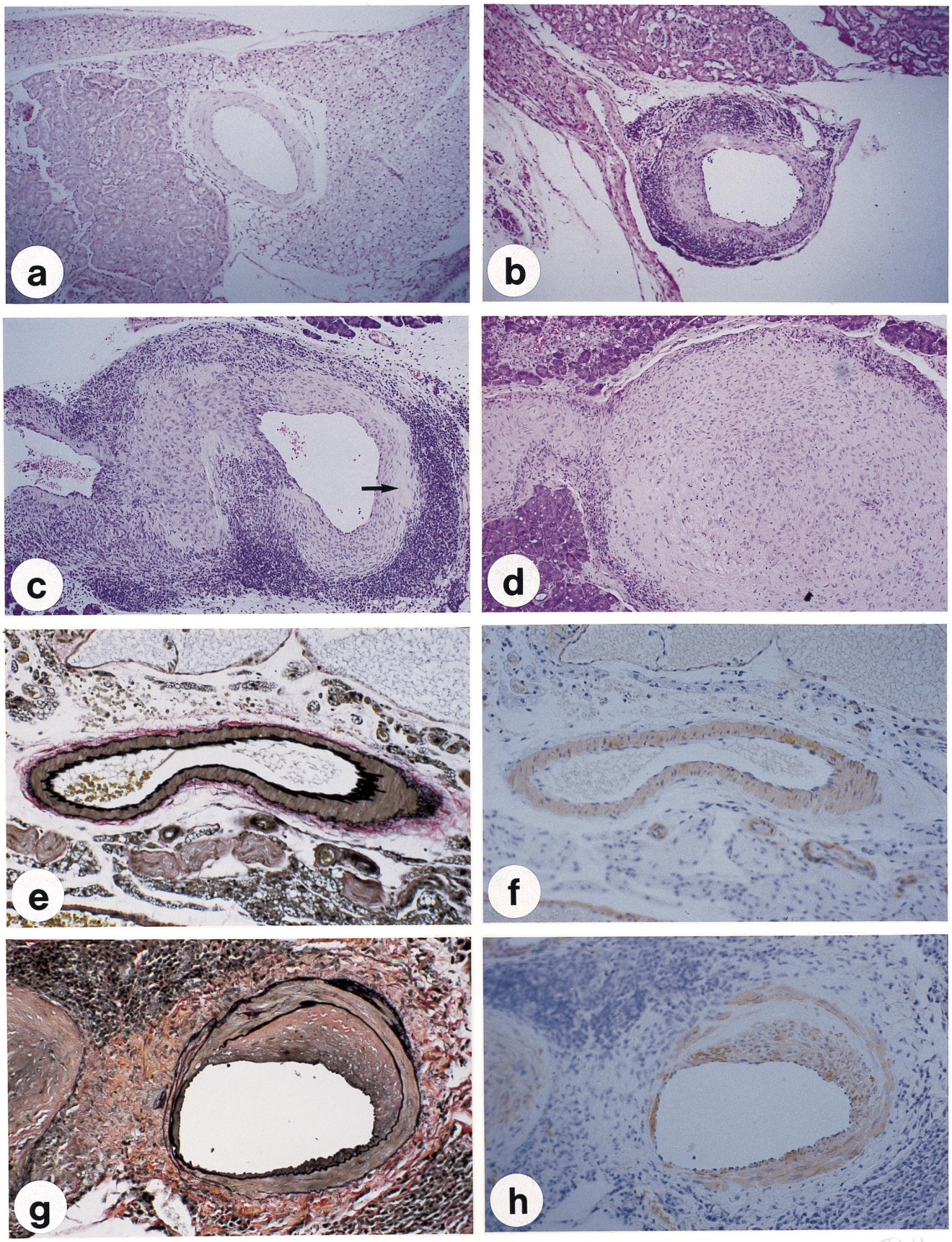
a
b
c
d
e
f
g
h

FIG. 2. Vascular lesions in HIV-1 transgenic mice. (a) Uninvolved renal artery from a nontransgenic mouse (hematoxylin and eosin; ×60). (b) Renal artery from a transgenic mouse showing extensive infiltration within the outer adventitia and disorganized SMCs (hematoxylin and eosin; ×60). (c) Mesenteric artery from the same transgenic mouse as in panel b showing extensive infiltration, disorganized SMCs with rounded vesicular nuclei, and an area of fibrosis (arrow) (hematoxylin and eosin; ×60). (d) Pancreatic artery from a different transgenic mouse that is occluded by SMC proliferation and exhibiting minimal immune cell infiltrates (hematoxylin and eosin; ×60). (e) Vessel from a nontransgenic control with intact IEL and EEL (Verhoeff's iron hematoxylin-van Geissan; ×120). (f) Serial section of vessel in panel e immunostained with an antibody against SMC α-actin (Mayer's hematoxylin counterstain; ×120). (g) Intimal thickening in a vessel from a different transgenic mouse (Verhoeff's iron hematoxylin-van Geissan; ×120). (h) Serial section of vessel in panel g immunostained with an antibody against SMC α-actin (Mayer's hematoxylin counterstain; ×120).

mune cell infiltration. Figure 2d depicts a pancreatic artery that is occluded primarily because of SMC proliferation. The apparent lack of fibrosis suggests that the hypertrophic growth is not the result of an inflammatory response but may actually precede it.

Both the internal and external elastic lamina (IEL and EEL, respectively [stained black]) which separate the three well-defined layers of the artery wall may be revealed by Verhoeff's iron hematoxylin-van Geissan staining of an uninvolved vessel from a nontransgenic mouse (Fig. 2e). The intima is composed of a single layer of endothelial cells and is delimited on its outer aspect by the IEL. The media consists of SMCs (stained brown) arranged in multiple lamellae and bounded on the luminal side by the IEL and on the abluminal side by the EEL. The adventitia, the outermost layer of the artery and delimited on the luminal aspect by the EEL, is made up of collagen bundles and elastic fibers (stained red). Immunostaining of a serial section with an antibody directed against SMC α-actin (13) shows the activity predominantly in the medial layer (Fig. 2f). A similar analysis of an involved vessel from a transgenic mouse reveals hypercellularity of the intimal layer on the luminal side of the IEL and hypocellularity in the medial layer (Fig. 2g). The hypercellular intima contains numerous α-actin-positive SMCs (Fig. 2h). This likely represents migration of SMCs from the media through the IEL and subsequent proliferation within the intima. The hypocellular area juxtaposed to the IEL is fibrotic because of collagen deposition (stained red). The EEL is somewhat fragmented and segmentally thickened. In other vessels, the fragmentation of the elastic lamina is overwhelming. The cellular infiltrate is localized distally to

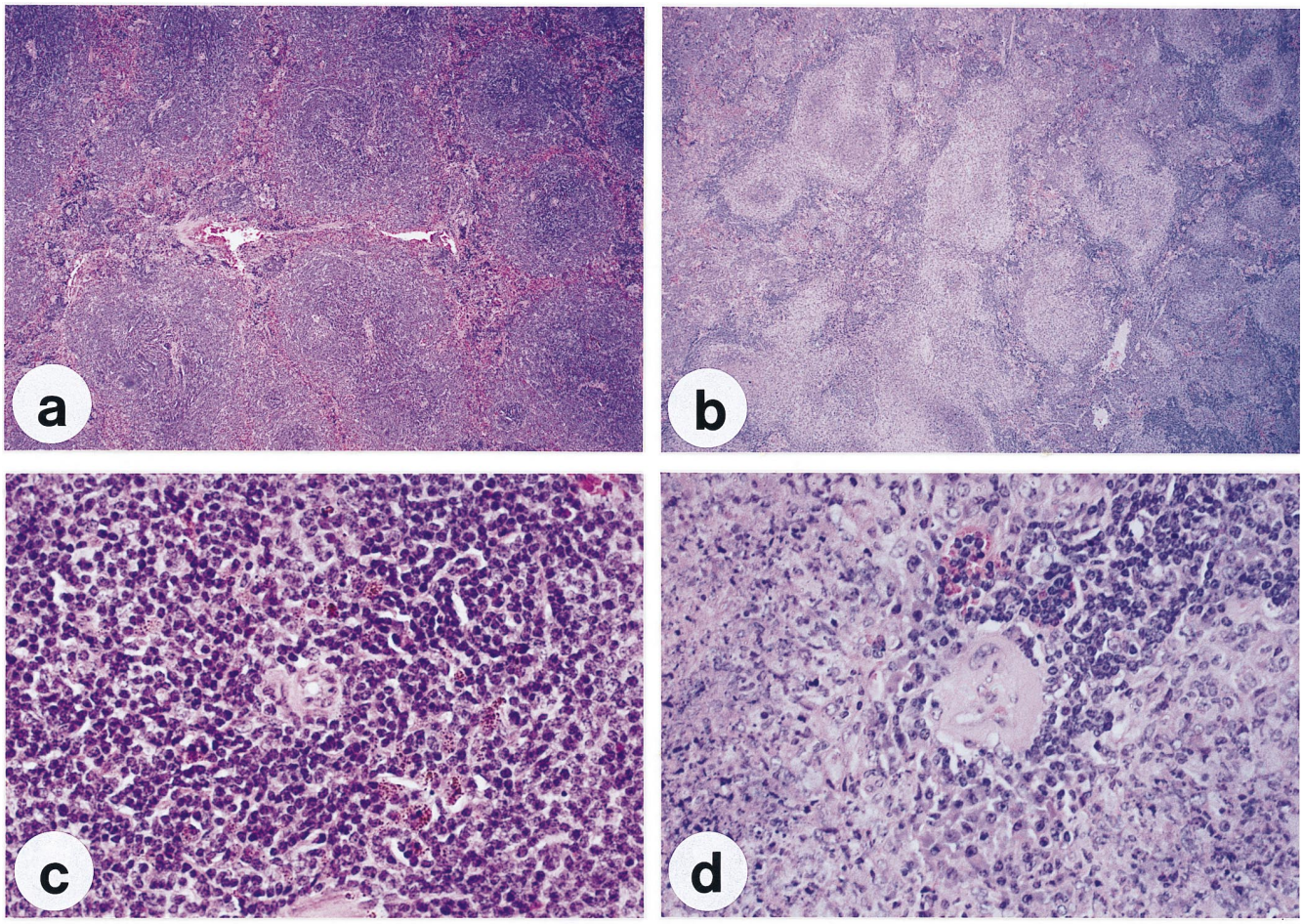

FIG. 3. Splenic infarcts in HIV-1 transgenic mice. (a) Low-magnification view of the spleen from a nontransgenic control mouse (hematoxylin and eosin; ×10). (b) Low-magnification view of the spleen from a transgenic mouse with splenic infarct (hematoxylin and eosin; ×10). (c) High-magnification view of a splenic white pulp with a central arteriole from a control mouse (hematoxylin and eosin; ×100). (d) High-magnification view of the white pulp area with an enlarged central arteriole from a transgenic mouse (hematoxylin and eosin, ×100).

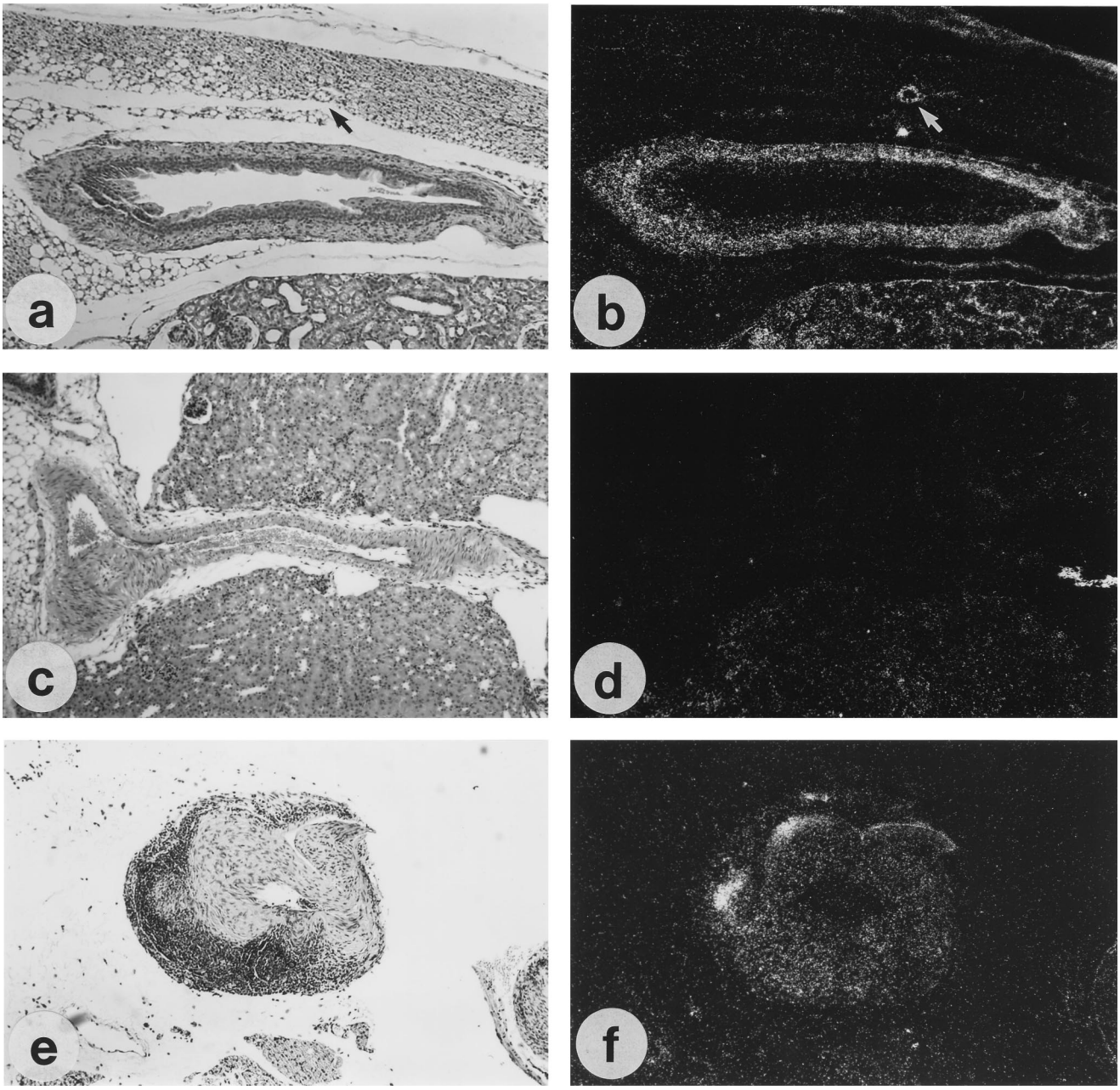

FIG. 4. HIV-1 gene expression in the blood vessel wall. Serial sections of blood vessels were either stained with hematoxylin and eosin and photographed under a bright field (a, c, and e) or subjected to in situ hybridization with an HIV-specific oligonucleotide probe and photographed under a dark field (b, d, and f). The oligonucleotide probe was specific for a sequence located within the U5 region of the LTR (5′-CCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGAC CC-3′) and was tailed with ^{35}S-dATP by using terminal deoxynucleotidyltransferase. (a and b) Artery from a transgenic mouse showing selective HIV gene expression in the media. Note the presence of a small vessel (arrow) embedded in the fat, overlying the major renal artery with intimal thickening. (c and d) Artery from a control mouse showing no detectable background hybridization with the HIV probe. (e and f) Artery from a transgenic mouse with extensive SMC proliferation showing no increase in hybridization signal over a region with abundant immune cell infiltration.

the EEL in the collagen-enriched adventitia. It is interesting to note that the endothelium remains intact and apparently uninvolved (Fig. 2h).

Such vascular changes are detected in a majority of the transgenic mice and may have significant functional consequences. Prolonged proliferation of SMCs may cause significant luminal narrowing, and restricted blood flow may result in ischemia. Indeed, some of the transgenic mice exhibited splenic infarcts, while other demonstrated evidence of renal thrombosis. For example, while the spleen of a nontransgenic mouse is composed of well-defined areas of white pulp (Fig. 3a), the spleen of a transgenic littermate exhibited extensive cell death in the same areas as a result of a splenic infarct (Fig. 3b). Unlike the central arteriole in the white pulp of the unaffected spleen (Fig. 3c), that in the transgenic mouse is enlarged and is surrounded by abundant cellular debris (Fig. 3d). Seemingly, the arteriopathy observed in the transgenic mouse resembles many of the clinical features seen among HIV-in-

fected patients (4, 6, 15, 16, 20, 21, 24, 34) and may be a complicating factor and even lethal (24).

The cell type responsible for initiating this series of changes in the vascular wall may be identified by an analysis of the site of HIV gene expression. In situ hybridization with an HIV probe demonstrates the accumulation of viral transcripts in the media of a vessel from a transgenic mouse (Fig. 4a and b), but not in a similar region from a control mouse (Fig. 4c and d). In the transgenic vessel wall, where there is no detectable immune infiltration but clear evidence of a neointima (Fig. 4a), hybridization is observed homogeneously throughout the medial layer and, to a lesser extent, the neointima (Fig. 4b). The restricted expression of HIV genes in SMCs correlates well with the proliferation of these cells in vivo. Analysis of a hypertrophic vessel with asymmetric immune cell infiltration (Fig. 4e) reveals viral transcripts in the resident SMCs but not in the infiltrating lymphocytes (Fig. 4f). This may suggest that infiltration of T cells is not likely to be the initiating event in this pathological change.

The analysis of two independent transgenic mouse lines, each carrying and expressing the HIV regulatory and accessory genes under the control of the HIV long terminal repeat (LTR), has revealed histopathological evidence of vasculopathy which is highly reminiscent of that detected in HIV-infected individuals (4, 6, 15, 16, 20, 21, 34). Our findings suggest that SMC proliferation is central to the observed changes in the blood vessel wall and is likely a consequence of HIV gene expression in these cells. The intimal and medial thickening can progress to significant luminal narrowing and tissue necrosis resulting from ischemia. It is likely that one or more of the HIV regulatory proteins is responsible for inducing the loss of growth arrest in the SMCs. Further studies to define the HIV gene(s) responsible for inducing the pathological changes will facilitate our understanding of the underlying biochemical mechanism. Implicit in our findings is the suggestion that the SMC is a direct target for HIV infection. Interestingly, HIV has been shown to infect a variety of cell types either through the CD4 receptor or by various non-CD4 mechanisms (10, 14, 23, 36). It will be important to determine whether HIV gene expression can be detected in the involved vessel walls in HIV-infected patients and if SMCs in culture can be infected by HIV.

The immune cell infiltration, characterized predominantly by T cells with occasional inclusion of B cells, may be secondary to the SMC hypertrophy. This suggestion is supported by our observation that SMC expansion was not always accompanied by immune cell infiltration and that HIV gene expression could not be detected in the infiltrating cells. The lack of granulocytes and macrophages in the infiltrate and the localization of infiltrating cells to the adventitia rather than the subendothelium most likely exclude immune complex deposition as a causative factor in this type of systemic vasculitis. However, these findings are not inconsistent with autoimmune disease in which T-cell infiltrates predominate.

This transgenic model may prove useful not only in the study of HIV-associated vasculopathy but also in our understanding of atherosclerosis. It has been hypothesized that lesions of atherosclerosis develop as a protective, inflammatory-fibroproliferative response to injury of the arterial wall (30). It has been speculated that insult to the endothelium results in an inflammatory response which involves the adherence of peripheral blood monocytes and T lymphocytes to the endothelium and invasion of the arterial wall by these cells. At these sites, activation of monocytes to macrophages results in the release of growth factors which can induce SMC migration and proliferation within the intima of the injured artery.

The SMC proliferation we observed differs significantly from the response-to-injury hypothesis in several ways. First, we observed no evidence for dysfunctional changes in the endothelium. The single layer of endothelial cells invariably remained intact and morphologically normal at the various stages of the disease process. Second, we detected no evidence for monocyte-derived macrophages. The infiltrate is composed of predominately T lymphocytes and some B cells and has not been detected in the subendothelium. Based upon these differences, the SMC proliferation in our transgenic mice is likely due to the activity of growth-inducing signals or changes indigenous to the SMCs which support HIV gene expression. Signaling from the endothelium or the macrophages does not appear to be part of the process.

While platelet-derived growth factor (9, 29, 31) (PDGF) and fibroblast growth factor (1, 3, 22, 28) (FGF) gene families are well recognized as key regulatory effectors of endothelial cell and SMC cell proliferation in vitro, their contribution to the hypertrophic events in this transgenic model is not known. Although PDGF is recognized as a mediator of SMC migration in vivo (12, 17), somatic gene transfer experiments have suggested that it is limited in its ability to promote vascular SMC hypertrophy in vivo (27). In contrast, the prominent angiogenic and SMC hypertrophic events mediated by a secretory form of FGF-1 following somatic gene transfer in vivo (25) suggest that members of the FGF gene family may be functional in the promotion of vascular SMC hypertrophy in vivo. Since the vascular SMC is potentially a rich source of FGF-1 (35, 37), it is possible that the function of the replication-defective HIV-1 provirus may be to influence the ability of FGF-1 to perform as an SMC mitogen.

We thank Lisa Ruiz for help in the preparation of the manuscript.

This work was supported by U.S. Public Health Service grant CA53633 to G.J.

REFERENCES

1. **Baird, A., and P. Böhlen.** 1990. Fibroblast growth factors, p. 369–418. *In* M. B. Sporn and A. B. Roberts (ed.), Handbook of experimental pharmacology: peptide growth factors and their receptors I. Springer-Verlag, Berlin, Germany.
2. **Bourgoignie, J. J., C. Oritz-Butcher, D. F. Green, and V. Pardo.** 1994. Renal disease and AIDS, p. 597–608. *In* S. Broder, T. C. Merigan, Jr., and D. Bolognesi (ed.), Textbook of AIDS medicine. Williams & Wilkins, Philadelphia, Pa.
3. **Burgess, W. H., and T. Maciag.** 1989. The heparin-binding (fibroblast) growth factor family of proteins. Annu. Rev. Biochem. **58:**575–606.
4. **Calabrese, L. H., M. Estes, B. Yen-Lieberman, M. R. Proffitt, R. Tubbs, A. J. Fishleder, and K. H. Levin.** 1989. Systemic vasculitis in association with human immunodeficiency virus infection. Arthritis Rheum. **32:**569–576.
5. **Chalifoux, L. V., M. A. Simon, D. R. Pauley, J. J. MacKey, M. S. Wyand, and D. J. Ringler.** 1992. Arteriopathy in macaques infected with simian immunodeficiency virus. Lab. Invest. **67:**338–349.
6. **Cho, E.-S., and L. R. Sharer.** 1990. Central nervous system in HIV infection, p. 43–63. *In* V. V. Joshi (ed.), Pathology of AIDS and other manifestations of HIV infection. Igaku-Shoin, New York, N.Y.
7. **Chu, Q. D., L. J. Medeiros, A. E. Fisher, R. F. Chaquette, and J. P. Crowley.** 1995. Thrombotic thrombocytopenic purpura and HIV infection. South. Med. J. **88:**82–88.
8. **del Arco, A., M. A. Martinez, J. M. Peña, C. Gamallo, J. J. Gonzàlez, F. J. Barbado, and J. J. Vazquez.** 1993. Thrombotic thrombocytopenic purpura associated with human immunodeficiency virus infection: demonstration of p24 antigen in endothelial cells. Clin. Infect. Dis. **17:**360–363.
9. **Deuel, T. F.** 1987. Polypeptide growth factors: roles in normal and abnormal cell growth. Annu. Rev. Cell. Biol. **3:**443–492.
10. **Fauci, A. S.** 1993. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. Science **262:**1011–1018.
11. **Feinberg, M. B., and W. C. Greene.** 1992. Molecular insights into human immunodeficiency virus type 1 pathogenesis. Curr. Opin. Immunol. **4:**466–474.
12. **Ferns, G. A. A., E. W. Raines, K. H. Sprugel, A. S. Motani, M. A. Reidy, and R. Ross.** 1991. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. Science **253:**1129–1132.
13. **Gabbiani, G., O. Kocher, W. S. Bloom, J. Vandekerckhove, and K. Weber.**

1984. Actin expression in smooth muscle cells of rat aortic intimal thickening, human atheromatous plaque, and cultured rat aortic media. J. Clin. Invest. **73:**148–152.
14. **Gallo, R. C., and L. Montagnier.** 1988. AIDS in 1988. Sci. Am. **259:**40–48.
15. **Gherardi, R., L. Belec, C. Mhiri, F. Gray, M.-C. Lescs, A. Sobel, L. Guillevin, and J. Wechsler.** 1993. The spectrum of vasculitis in human immunodeficiency virus-infected patients. Arthritis. Rheum. **36:**1164–1174.
16. **Gherardi, R., C. Mhiri, M. Baudrimont, E. Roullet, J.-P. Berry, and J. Poirier.** 1991. Iron pigment deposits, small vessel vasculitis, and erythrophagocytosis in the muscle of human immunodeficiency virus-infected patients. Hum. Pathol. **22:**1187–1194.
17. **Heldin, C.-H., and B. Westermark.** 1990. Platelet-derived growth factor: mechanism of action and possible *in vivo* function. Cell Regul. **1:**555–566.
18. **Holland, G. N.** 1994. Ocular sequelae, p. 473–487. *In* S. Broder, T. C. Merigan, Jr., and D. Bolognesi (ed.), Textbook of AIDS medicine. Williams & Wilkins, Philadelphia, Pa.
19. **Jorda, M., M. M. Rodriguez, and R. A. Reik.** 1994. Thrombocytic thrombocytopenic purpura as the cause of death in an HIV-positive child. Pediatr. Pathol. **14:**919–925.
20. **Joshi, V. V., B. Pawel, E. Conner, L. Sharer, J. M. Oleske, S. Morrison, and J. Marin-Garcia.** 1987. Arteriopathy in children with acquired immune deficiency syndrome. Pediatr. Pathol. **7:**261–275.
21. **Kabus, D., and M. A. Greco.** 1991. Arteriopathy in children with AIDS: microscopic changes in the vasa vasorum with gross irregularities of the aortic limb. Pediatr. Pathol. **11:**793–795.
22. **Klagsbrun, M., and E. R. Edelman.** 1989. Biological and biochemical properties of fibroblast growth factors. Implications for the pathogenesis of atherosclerosis. Arteriosclerosis **9:**269–278.
23. **Levy, J. A.** 1993. Pathogenesis of human immunodeficiency virus infection. Microbiol. Rev. **57:**183–289.
24. **Mezzaroma, I., C. Carini, A. Cirelli, and F. Aiuti.** 1987. HIV infection, vasculitis and immune complexes. AIDS **1:**131–132.
25. **Nabel, E. G., Z.-Y. Yang, G. Plautz, R. Forough, X. Zhan, C. C. Haudenschild, T. Maciag, and G. J. Nabel.** 1993. Recombinant fibroblast growth factor-1 promotes intimal hyperplasia and angiogenesis in arteries *in vivo*. Nature **362:**844–846.
26. **Pepose, J. S., G. N. Holland, M. S. Nestor, A. J. Cochran, and R. Y. Foos.** 1985. Acquired immune deficiency syndrome: pathogenic mechanisms of ocular disease. Ophthalmology **92:**472–484.
27. **Pompili, V. J., D. Gordon, H. San, Z. Yang, D. W. M. Muller, G. J. Nabel, and E. G. Nabel.** 1995. Expression and function of a recombinant PDGF B gene in porcine arteries. Arterioscler. Thromb. Vasc. Biol. **15:**2254–2264.
28. **Presta, M., D. Moscatelli, J. Joseph-Silverstein, and D. B. Rifkin.** 1986. Purification from a human hepatoma cell line of a basic fibroblast growth factor-like molecule that stimulates capillary endothelial cell plasminogen activator production, DNA synthesis, and migration. Mol. Cell. Biol. **6:**4060–4066.
29. **Raines, E. W., D. F. Bowen-Pope, and R. Ross.** 1990. Platelet-derived growth factor, p. 173–262. *In* M. B. Sporn and A. B. Roberts (ed.), Handbook of experimental pharmacology: peptide growth factors and their receptors I. Springer-Verlag, Berlin, Germany.
30. **Ross, R.** 1993. Atherosclerosis: a defense mechanism gone awry. Am. J. Pathol. **143:**987–1002.
31. **Ross, R., E. W. Raines, and D. F. Bowen-Pope.** 1986. The biology of platelet-derived growth factor. Cell **46:**155–169.
32. **Stroncek, D. F., W. E. Kline, M. E. Clay, L. B. Plachta, R. B. Stricker, H. Hollander, J. S. Greenspan, and K. M. Skubitz.** 1992. Antibodies to granulocytes in patients infected with human immunodeficiency virus. J. Lab. Clin. Med. **119:**724–731.
33. **Tinkle, B. T., H. Ueda, L. Ngo, P. A. Luciw, K. Shaw, C. A. Rosen, and G. Jay.** Transgenic dissection of HIV genes involved in lymphoid depletion. J. Clin. Invest., in press.
34. **Valeriano-Marcet, J., L. Ravichandran, and L. D. Kerr.** 1990. HIV associated systemic necrotizing vasculitis. J. Rheumatol. **17:**1091–1093.
35. **Weich, H. A., N. Iberg, M. Klagsbrun, and J. Folkman.** 1990. Expression of acidic and basic fibroblast growth factors in human and bovine vascular smooth muscle cells. Growth Factors **2:**313–320.
36. **Weiss, R. A.** 1994. The virus and its target cells, p. 15–20. *In* S. Broder, T. C. Merigan, Jr., and D. Bolognesi (ed.), Textbook of AIDS medicine. Williams & Wilkins, Philadelphia, Pa.
37. **Winkles, J. A., and C. G. Gay.** 1991. Serum, phorbol ester, and polypeptide mitogens increase class 1 and 2 heparin-binding (acidic and basic fibroblast) growth factor gene expression in human vascular smooth muscle cells. Cell Growth Differ. **2:**531–540.