Original article

Folia Neuropathologica

Complex immune-mediated mechanisms of vasculitis in cerebral toxoplasmosis in AIDS patients

Dorota Dziewulska[1,2], Janina Rafałowska[1]

[1]Department of Experimental and Clinical Neuropathology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, [2]Department of Neurology, Medical University of Warsaw, Poland

Folia Neuropathol 2014; 52 (1): 86-91 DOI: 10.5114/fn.2013.39721

Abstract

The increasing population of patients treated with immunosuppressive and immunomodulatory drugs, as well as growing resistance to anti-retroviral therapy, has caused the reemergence of cerebral toxoplasmosis as a clinical problem. Encephalitis caused by Toxoplasma gondii (Tg) is the most common opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS). The scarcity of data concerning vessel involvement in cerebral toxoplasmosis in AIDS impelled us to examine this process.

In 15 of 178 cases with AIDS cerebral toxoplasmosis was the only opportunistic infection. In these patients routine histological stains and immunohistochemical reactions against T and B lymphocytes, immunoglobulins IgG and IgM, and factors C4β and B, involved respectively in classical and alternative pathways of complement activation, were performed. Apart from morphological changes typical for cerebral toxoplasmosis, eosinophilic necrosis of the vascular media, and vascular inflammatory infiltrates containing T and B lymphocytes were seen. In some arterial vessels intramural deposits of immunoglobulin IgG and IgM, and complement factors C4β and B were found.

Presence of polyarteritis nodosa-like changes, deposits of immunoglobulins and complement factors in the vessel wall, as well as inflammatory infiltrates containing B lymphocytes indicate that vasculitis in cerebral toxoplasmosis in AIDS has a very complex pathomechanism involving not only cell-mediated but also humoral-mediated immunological reactions.

Key words: *AIDS, complement, HIV, toxoplasmosis, vasculitis.*

To the memory of Prof. Mirosław M. Mossakowski, who, together with Prof. Irmina Zelman, gathered a significant AIDS Brain Collection in the Mossakowski Medical Research Centre.

Introduction

Toxoplasma gondii (Tg) infection in immunocompetent humans is generally rare and latent. But persistent cyst containing latent forms of Tg may be responsible for reactivation of the toxoplasmic infection. Although the process of Tg reactivation is poorly understood, it is known that Tg cysts are controlled by the intact immune system, and only in the case of immune suppression CD4+ T cells are unable to suppress this latent infection. That dysfunction leads to the release of bradyzoites from cysts, their conver-

Communicating author:

Dorota Dziewulska, MD, PhD, Department of Neurology, Medical University of Warsaw, Banacha 1 A, 02-097 Warsaw, Poland, phone: +48 22 599 28 75, fax: +48 22 599 18 57, e-mail: dorota.dziewulska@wum.edu.pl

sion to tachyzoites and toxoplasmosis reactivation. Some studies suggest that dendritic cells may act as Trojan horses to spread the infection [4,13]. An alternative hypothesis assumes that cyst rupture and re-formation of a new cyst is a constant process even in immunocompetent individuals, and the role of the immune system is limited to the control of the tachyzoites [7].

Apart from brain damage, a very common result of Tg invasion is ocular involvement. The infection affecting various parts of the eyes was described both in humans [1,16,17,20] and in experimental animals [21,22]. While in immunocompetent individuals Tg infection is frequently asymptomatic, in immunocompromised patients toxoplasmic encephalitis, retinochoroiditis, and uveitis are frequent [16]. Encephalitis caused by Tg is the most common opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS) and has been well characterized in the literature. But cerebral vasculitis in patients with toxoplasmosis and AIDS was described only occasionally and mainly in case reports from the 1980s [3,10].

Many components of the host immune response to Tg such as production of gamma interferon, cell-mediated immunity, and control of Tg replication by macrophages participate in development of toxoplasmic encephalitis but the exact pathomechanism of Tg neuroinfection in AIDS patients is poorly understood, especially the pathomechanism of vasculitis. But not only the scarcity of data concerning vessel involvement in Tg infection in AIDS impelled us to examine this process. Growing drug resistance to anti-retroviral therapy and the increasing population of patients treated with immunosuppressive or immunomodulatory drugs caused the reemergence of cerebral toxoplasmosis as a clinical problem.

Material and methods

In the AIDS Brain Collection of the Mossakowski Medical Research Centre 178 brains from AIDS patients were collected. Among them cerebral toxoplasmosis was diagnosed in 32 cases (18%) [16]. In 15/32 cases cerebral toxoplasmosis was the only opportunistic infection and these brains were selected for further investigations. These patients died at the age of 30-40 years and one at the age of 52 years. The investigated material was collected between 1992 and 1998 and in none of the patients highly active antiretroviral therapy (HAART) or immunomodulatory management was applied.

On formalin-fixed and paraffin-embedded tissue samples from cerebral hemispheres routine histological stains (hematoxylin and eosin [H&E], Klüver-Barrera method), and immune reactions were applied. Immunohistochemistry was performed using routine microwave antigen retrieval and avidin-biotin-peroxidase methods with antibodies against: *Toxoplasma gondii* (Novocastra, NCL-TG, 1 : 15), fibronectin (Santa Cruz Biotechnology, sc-69681, 1 : 100), IgG (DAKO, A0423, 1 : 500), IgM (DAKO, A0425, 1 : 500), B lymphocyte marker (Novocastra, NCL-MB2, 1 : 50), and T lymphocytes: CD3 (Novocastra, NCL-CD3-PS1 1 : 100), and CD8 (Novocastra, NCL-CD8-295, 1 : 20). To define involvement of the complement system in vasculitis we performed immunohistochemical reactions with antibodies against factor C4β (Santa Cruz Biotechnology, sc-25816, 1 : 250) participating in the classical pathway of complement activation and factor B (Santa Cruz Biotechnology, sc-67141, 1 : 500) involved in the alternate pathway.

Results

In all examined brains Tg cysts or tachyzoites were found. They were visible in brain parenchyma (Fig. 1A), vessel lumen (Fig. 1B) or vessel wall. Apart from different forms of the parasite, numerous foci of coagulative necrosis ("toxoplasmic abscesses") of different size and location were observed. Within the necrotic foci, vessels with the lumen occluded by thrombosis were often visible. Some of the necrotic foci revealed an advanced stage of the breakdown process and they were surrounded by inflammatory infiltrates (granulomatous ridge) composed of leukocytes, lymphocytes, phagocytic cells, histiocytes, and infrequent plasmatic cells. In granulomatous ridge and nearby parenchyma, inflammatory infiltrates were visible both in the perivascular space and within the walls of arteries (Fig. 1C) and veins (Fig. 1D). Distinct inflammation was also found in tissue distant from the necrotic lesions but in such areas inflammatory infiltrates were visible only in precapillary and capillary blood vessels (Fig. 1E). In routine histopathological assessment, vessel inflammation did not demonstrate any characteristic features distinct from vasculitides observed in other neuroinfections.

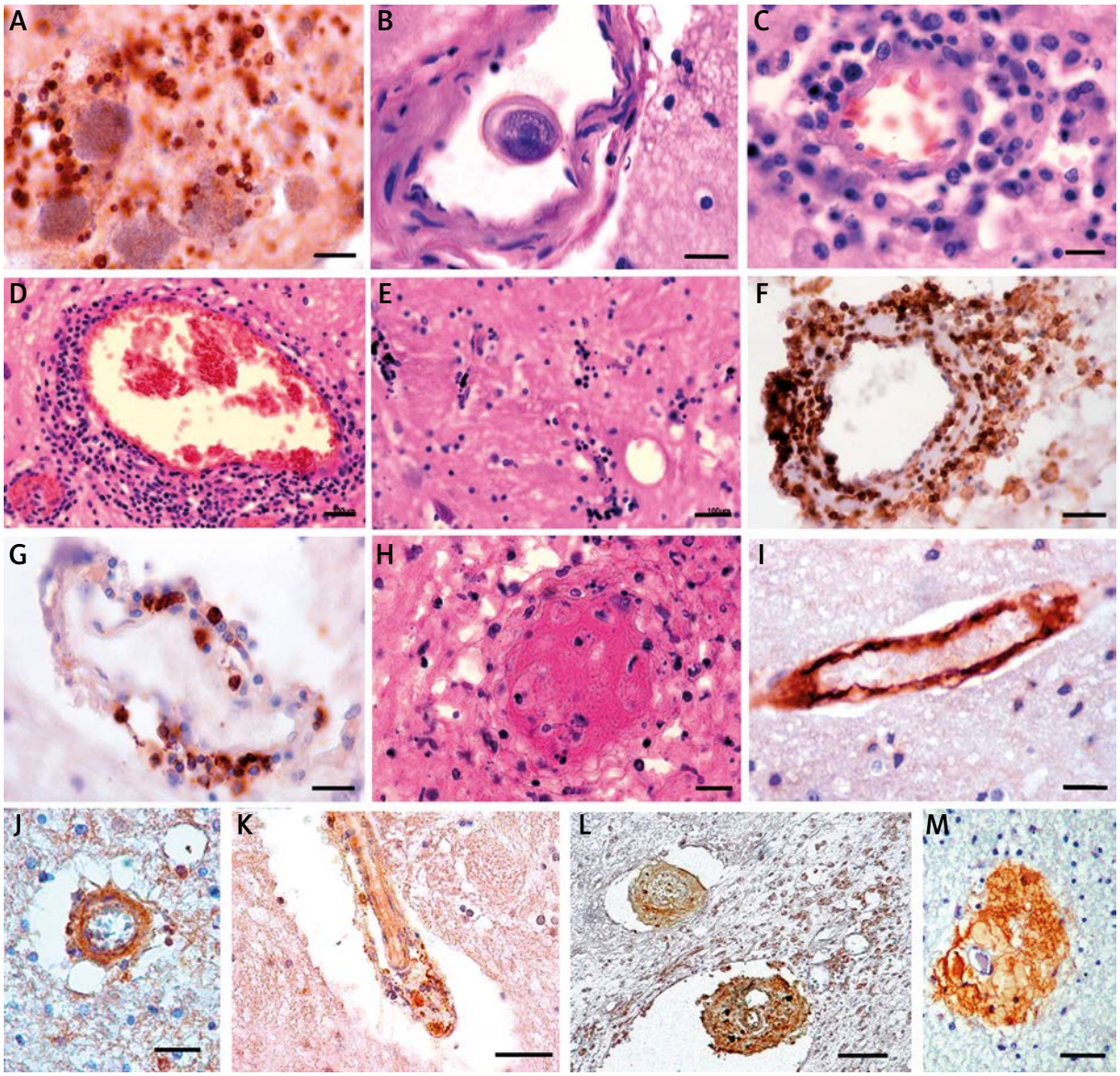

Fig. 1. A) Tachyzoites in brain parenchyma, anti-Tg, bar – 100 µm. **B)** Toxoplasmic cyst in vessel lumen, H&E, bar – 100 µm. **C)** Small cerebral artery infiltrated by mononuclear cells, H&E, bar – 100 µm. **D)** Small cerebral vein with inflammatory infiltrates, H&E, bar – 200 µm. **E)** Infiltrates around precapillary and capillary blood vessels, H&E, bar – 200 µm. **F)** Numerous CD3+ T lymphocytes in vessel wall infiltrates, anti-CD3, bar – 100 µm. **G)** Non-numerous B lymphocytes in vessel wall infiltrates, anti-B lymphocyte marker, bar – 100 µm. **H)** Eosinophilic fibrinoid necrosis of the vascular media with occlusion of the vessel lumen, H&E, bar – 100 µm. **I)** Strong positive immune reaction to IgG in wall of small cerebral artery, anti-IgG, bar – 100 µm. **J)** Moderately positive immune reaction to IgM in vessel wall, anti-IgM, bar – 100 µm. **K)** Intramural deposits of factor C4β in wall of the white matter artery; anti-C4β, bar – 200 µm. **L)** Granular immunolabel for complement factor B in wall of small arterial vessels; visible moderately positive cytoplasmic reaction in parenchymal macrophages; anti-factor B, bar – 250 µm. **M)** Immunolabel for fibronectin around small vessel in the cerebral white matter, anti-fibronectin, bar – 200 µm.

Immunohistochemical reactions revealed predominance of CD3+ T lymphocytes in inflammatory infiltrates localized both in granulomatous ridge and in vessel wall (Fig. 1F) while CD8+ T lymphocytes and B lymphocytes were found infrequently and only within vessel wall (Fig. 1G). In five cases, apart from intramural inflammatory infiltrates, eosinophilic fibrinoid necrosis of the vascular media was observed (Fig. 1H). In some of the cerebral blood vessels immunopositive reactions to IgG (Fig. 1I) and IgM (Fig. 1J) were observed. Deposits of the immunoglobulins were observed in the walls of medium- and small-sized arterial vessels but not in veins and capillaries. The immune reactions for compounds of the complement system had a granular character and they were moderately positive for factor C4β (Fig. 1K) and strongly for factor B (Fig. 1L). Positive immunolabel for C4β was observed only in some of the parenchymal small arteries while a pronounced immune reaction for factor B was seen in numerous vessels of different types. Immunoreactivity for both complement factors was also noted in macrophages. In some cases with coagulative necrosis increased perivascular immunolabel for both complement compounds and fibronectin (Fig. 1M) was found.

The vascular changes revealed in immunohistochemical reactions and described above were observed in vessels not only localized within foci of coagulative necrosis and granulomatous ridge but also in nearby parenchyma but not in distant precapillaries or capillaries with inflammatory infiltrates.

Discussion

Our investigation on cerebral toxoplasmosis in AIDS patients showed, typically for the disease, morphological foci of coagulative necrosis and inflammation involving cerebral vessels.

In ischemic vascular diseases coagulative necrosis due to stenosis or occlusion of the vessel lumen is rarely observed because this stage of necrosis is usually transient. Normally, after the onset of ischemia, damage to the blood-brain barrier (BBB) leads to increased permeability of the vessel wall and afflux of leukocytes, lymphocytes and monocytes/macrophages. That phenomenon results in tissue breakdown and development of colliquative necrosis. In AIDS and other immunocompromised patients, severe dysfunction of the immune system can delay tissue breakdown and maintenance of the coagulative changes.

Although in HIV encephalitis perivascular infiltrates disseminated in brain parenchyma are common, involvement of the vessel wall is very rare with an incidence of less than 1% [9]. In our material intramural inflammatory infiltrates were observed in vessels of different type and size localized not only within foci of coagulative necrosis but also in adjacent and distant parenchyma. The affected vessels sometimes revealed perivascular immunoexpression of fibronectin and complement factor C4β. Since complement factor C4β and a fraction of fibronectin exist in a soluble form in serum, their perivascular occurrence indicates increased permeability of the vessel wall. This phenomenon characteristic for disturbed BBB may play a key role in Tg spread in the CNS [7,12].

Apart from intramural inflammatory infiltrates, some cerebral vessels also revealed eosinophilic necrosis of the muscular layer. Eosinophilic necrosis in cerebral vessels has been observed in many different diseases from idiopathic arterial hypertension to amyloid angiopathy. However, in such patients the vessel wall was usually devoid of inflammatory infiltrates.

It is still unknown what factor(s) is directly responsible for development of vasculitis in cerebral toxoplasmosis in AIDS. It is known that in ischemic vascular diseases presence of coagulative or colliquative necrosis does not lead to vasculitis. Therefore other factors have to be responsible for vessel inflammation. There are two major mechanisms by which infection can induce vasculitis: (1) direct invasion of the microorganism due to damage to the vessel wall, and (2) immune-mediated injury.

In cerebral toxoplasmosis both HIV and Tg can give rise to development of vasculitis. In HIV positive individuals without co-infections with other pathogens, a wide spectrum of vasculitides has been found [9]. But presence of Tg cysts in vessel lumen and tachyzoites in vascular wall can also cause vessel damage, particularly damage to endothelial cells, and triggers not only thrombotic cascade [10] but also inflammatory and immune reactions. Results of recent investigations confirmed that Tg can modulate gene expression of cerebral endothelial cells to promote its own migration through the BBB [12]. Among these modulatory changes, prominent upregulation of the cell adhesion molecule ICAM-1, activated leukocyte cell adhesion molecule (ALCAM), and vascular cell adhesion molecule 1 (VCAM-1) was

found [19]. Expression of different classes of adhesion molecules observed both in acute and chronic toxoplasmic encephalitis [5] not only facilitates Tg migration but also supports infiltration of the vessel wall by circulating blood cells and development of inflammation.

Another pathomechanism implicated in the induction of vasculitis is immune-mediated inflammation in which vessel damage may be caused by cell-mediated, immune complex-mediated and autoantibody-mediated reactions.

T cell-mediated vascular injury has been demonstrated in several types of vasculitides including PAN and AIDS. It is known that in HIV infection there is an oligoclonal expansion of T cells, especially CD8 + cells [8]. But our and some other authors' immunohistochemical studies revealed that lymphocytes seen in the vessel wall and perivascular space are not exclusively of the T but also of B cell lineage.

In our material some of the arterial vessels showed intramural deposits of IgG and IgM. Although immunoglobulin deposits in the vessel wall have been described in many disorders, even in some genetically determined vascular diseases such as CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) [18], they are highly indicative for the immune complex-mediated pathomechanism of vasculitis. In patients with Tg infection, specific IgM, IgG, IgA, and even IgE antibodies were found [6,15]. In immunocompetent individuals with latent Tg infection, the parasites inside the brain may be protected from circulating antibodies by intact BBB [14]. But in immunodepressed patients with toxoplasmosis reactivation, damage to the BBB may promote antibody accumulation in the vessel wall leading to development of vasculitis.

Vasculitis induced by the immune complexes is identified by the presence of immunoglobulin and complement deposits in the vessel wall [11]. In our material not only deposits of IgG and IgM were found but also complement factors C4β and B.

Complement is activated by three pathways: the classical, the alternative and the lectin pathway. Factor C4β is a potent anaphylatoxin that is released during the classical pathway of complement activation while factor B is involved in the alternative pathway. It is known that in the classical pathway, the initiator complex is activated by a wide range of targets, including apoptotic cells, some viruses and bacteria, and antibody-antigen complexes containing immunoglobulin IgG or IgM [2]. The initiation of the alternative pathway does not require presence of the immune complexes but starts with spontaneous hydrolysis of the C3 component or by its binding to specific targets on microorganism and eukaryotic cells. The lectin pathway is also independent of the immune complexes and is initiated by lectin binding to moieties localized on the surface of microorganisms or dying cells. The intramural deposits of immunoglobulins and complement factor C4β in vessels observed by us suggest that complement activation via the classical pathway is involved in development of vasculitis. But the positive immune reaction for factor B in some vessels indicates that complement may also be activated via the alternative pathway which is less specific but more autoaggressive than the classical one.

Vasculitis in AIDS patients with cerebral toxoplasmosis is a very complex system where many factors are unbalanced. Although it is difficult to establish certainly whether toxoplasmosis itself leads to the above-described vascular changes, in the presented study we demonstrated that several distinct immune-mediated mechanisms may coexist in the vasculitis and therefore finding an efficient therapy is still a challenge.

References

1. Antoniazzi E, Guagliano R, Meroni V, Pezzotta S, Bianchi PE. Ocular impairment of toxoplasmosis. Parassitologia 2008; 50: 35-36.
2. Carroll MC. The complement system in regulation of adaptive immunity. Nat Immunol 2004; 5: 981-986.
3. Chaudhari AB, Singh A, Jindal S, Poon TP. Haemorrhage in cerebral toxoplasmosis. A report on a patient with the acquired immunodeficiency syndrome. S Afr Med J 1989; 76: 272-274.
4. Courret N, Darche S, Sonigo P, Milon G, Buzoni-Gâtel D, Tardieux I. CD11c- and CD11b-expressing mouse leukocytes transport single Toxoplasma gondii tachyzoites to the brain. Blood 2006; 107: 309-316.
5. Deckert-Schlüter M, Schluter D, Hof H, Wiestler OD, Lassmann H. Differential expression of ICAM-1, VCAM-1 and their ligands LFA-1, Mac-1, CD43, VLA-4, and MHC class II antigens in murine Toxoplasma encephalitis: a light microscopic and ultrastructural immunohistochemical study. J Neuropathol Exp Neurol 1994; 53: 457-468.
6. Dziubek Z, Zarnowska H, Basiak W, Gorski A, Kajfasz P. Some aspects of immune response in toxoplasmosis. Przegl Epidemiol 2001; 55: 495-502.
7. Feustel SB, Meissner M, Liesenfeld O. Toxoplasma gondii and the blood-brain barrier. Virulence 2012; 3: 182-192.

8. Gisselbrecht M, Cohen P, Lortholary O, Jarrousse B, Gayraud M, Lecompte I, Ruel M, Ghérardi R, Guillevin L. Human immunodeficiency virus-related vasculitis. Clinical presentation of and therapeutic approach to eight cases. Ann Med Interne (Paris) 1998; 149: 398-405.
9. Guillevin L. Vasculitides in the context of HIV infection. AIDS 2008; 22 Suppl 3: S27-33.
10. Huang TE, Chou SM. Occlusive hypertrophic arteritis as the cause of discrete necrosis in CNS toxoplasmosis in the acquired immunodeficiency syndrome. Hum Pathol 1988; 19: 1210-1214.
11. Jennette JCh. Implications for pathogenesis of patterns of injury in small- and medium-sized-vessel vasculitis. Cleve Clin J Med 2002; 69 Suppl 2: SII33-38.
12. Lachenmaier SM, Deli MA, Meissner M, Liesenfeld O. Intracellular transport of Toxoplasma gondii through the blood-brain barrier. J Neuroimmunol 2011; 232: 119-130.
13. Lambert H, Hitziger N, Dellacasa I, Svensson M, Barragan A. Induction of dendritic cell migration upon Toxoplasma gondii infection potentiates parasite dissemination. Cell Microbiol 2006; 8: 1611-1623.
14. Masocha W, Kristensson K. Passage of parasites across the blood-brain barrier: Virulence 2012; 3: 202-212.
15. Matowicka-Karna J, Dymicka-Piekarska V, Kemona H. Does Toxoplasma gondii infection affect the levels of IgE and cytokines (IL-5, IL-6, IL-10, IL-12, and TNF-alpha)? Clin Dev Immunol 2009; 2009: 374696; doi: 10.1155/2009/374696.
16. Mossakowski MJ, Zelman IB. Neuropathological syndromes in the course of full blown acquired immune deficiency syndrome (AIDS) in adults in Poland (1987-1995). Folia Neuropathol 1997; 35: 133-143.
17. Pleyer U, Torun N, Liesenfeld O. Ocular toxoplasmosis. Ophthalmologe 2007; 104: 603-615.
18. Rafałowska J, Fidzianska A, Dziewulska D, Podlecka A, Szpak GM, Kwiecinski H. CADASIL: new cases and new questions. Acta Neuropathol 2003; 106: 569-574.
19. Silva NM, Manzan RM, Carneiro WP, Milanezi CM, Silva JS, Ferro EA, Minor JR. Toxoplasma gondii: the severity of toxoplasmic encephalitis in C57BL/6 mice is associated with increased ALCAM and VCAM-1 expression in the central nervous system and higher blood-brain barrier permeability. Exp Parasitol 2010; 126: 167-177.
20. Smith JR, Cunningham ET Jr. Atypical presentations of ocular toxoplasmosis. Curr Opin Ophthalmol 2002; 13: 387-392.
21. Stahl W, Turek G. Chronic murine toxoplasmosis: clinicopathologic characterization of a progressive wasting syndrome. Ann Trop Parasitol 1988; 82: 35-48.
22. Webb RM, Tabbara KF, O'Connor GR. Retinal vasculitis in ocular toxoplasmosis in nonhuman primates. Retina 1984; 4: 182-188.