Granulomatous amoebic encephalitis: ghost response of an immunocompromised host?

Naegleria fowleri, Acanthamoeba and Balamuthia mandrillaris spp. are known to cause fatal amoebic encephalitis. Here, I attempt to draw attention to these cases, which are reported as ‘granulomatous amoebic encephalitis’ (GAE), and their occurrence in immunocompromised individuals and patients with AIDS. GAE, like any other granulomatous inflammation, can occur only in the presence of ample numbers of CD4+ T-lymphocytes. Extensive reviews of manuscripts published over a period of 50 years on this topic and cytokine studies and/or morphological evidence provided in peer-reviewed published studies were evaluated in detail by independent resources to analyse the granulomatous inflammatory evidence provided to justify the title of GAE in this group of patients. The evidence given in support of GAE did not appear to be convincing enough in the majority of published studies, and in particular its occurrence in patients with AIDS and other immunocompromised states was not justified. The distinction between the early development of type IV hypersensitivity reactions and granuloma/ granulomatous inflammation was found to be vague and inconclusive. It is therefore recommended that this terminology is used only when all the diagnostic criteria have been met, and use of a term such as ‘granulomatoid’ is suggested in cases where there remains an ambiguity in the morphological appearance of the lesions, especially in AIDS and related diseases.

Free-living amoebas that are pathogenic protists, such as Naegleria fowleri, Balamuthia mandrillaris and Acanthamoeba spp., are known to cause central nervous system infections termed encephalitis in humans, with the first report of amoebic encephalitis caused by amoeba dating back to the 1950s (Guibert & Ballard, 1959). The inflammatory host response to B. mandrillaris and pathogenic Acanthamoeba spp. protists is known as ‘granulomatous amoebic encephalitis’ (GAE) (Seijo Martinez et al., 2000; Silva et al., 2010); when caused by N. fowleri, the term primary amoebic meningoencephalitis (PAM) (Guarner et al., 2007) is used in the literature. GAE as a term was first reported to be the host response that occurs in chronically ill patients and patients with immunodeficiency, and these cases may present as an acute or subacute intracerebral mass lesion with signs and symptoms of focal brain disease (Martinez et al., 1980). Several years prior to the first use of the term ‘granulomatous’ for encephalitis caused by Balamuthia and Acanthamoeba spp., published studies were very clearly advocating the cardinal role of T-cell immunity in these immune variants of granulomatous inflammations (Unanue & Benacerraf, 1973) and the notion that, in its absence, the lesions do not progress to a granulomatous appearance. In the years that followed the appearance of this nomenclature, usage of the term GAE became very common and started appearing in many parasitology and immunopathology journals and text books, with a worrying aspect to it, which was, and still is, its use with regard to immunocompromised patients (Seijo Martinez et al., 2000; Marciano-Cabral et al., 2000; Vivesvare, 2013), such as those with AIDS (Zagardo et al., 1997; Pietrucha-Dilanchian et al., 2011), who have substantial T-cell depletion, one reason why this disease got its name in the first place.

Newer contributions towards our understanding of immune responses resulting in granulomatous inflammatory lesions have led to a considerable increase in the number of studies that have reported its occurrence in a number of protist infections. With ongoing efforts to understand this immune response, there has been an increase in the inclination to use new abbreviations and terminologies for lesions akin to granulomatous inflammations. Further confusion comes from other reports for protists such as a B. mandrillaris infection in a human immunodeficiency virus-infected patient that was reported as GAE, caused by leptomyxid amoebas, with conclusions and results referring to the absence of granulomatous lesions (Zagardo et al., 1997) while stressing that human immunodeficiency virus patients with normal CD4+ counts should be suspected of GAE, especially if caused by a leptomyxid amoeba. A solitary or mixture of findings of perivascular cuffing (seen mostly in the early development of type IV hypersensitivity reactions, but not in granuloma/granulomatous inflammation), appearances of inflammatory giant cells alone, fibrinoid necrosis, vasculitis and necrotic debris with stainable trophozoites (Pietrucha-Dilanchian et al., 2012) and cysts (Guarner et al., 2007; Silva et al., 2010) in the midst of the lesions have been ascribed to GAE. However, evidence based on related cytokine and/or morphologically diagnostic epithelioid cells, with or without inflammatory giant cells if made more apparent, might have been more convincing in studies with the title of ‘granulomatous amoebic encephalitis’ in AIDS and other related immunodeficient patients.

Validation of a true granulomatous inflammatory lesion requires morphological and/or cytokine detection that includes the presence of epithelioid cells, usually surrounded by a rim of lymphocytes with or without inflammatory giant cells (Fig. 1), and is associated with a strong CD4+ T-cell (Th1) activation and cytokine (IFN-γ, TNF, IL-2 and IL-12) production. The formation of an early perivascular cuffing by inflammatory cells occurs within 24–72 h in delayed type IV hypersensitivity reactions and such lesions commonly present as perivascular cellular cuffing with inflammatory cells at or around the lesion, while granulomatous inflammation tends to develop within 2–3 weeks; both require
a strong T-cell response for their development (Kumar et al., 2010) (Fig. 2). Moreover, one of the consistent features of the diagnostic epithelioid cell is the virtual absence of recognizable endocytosed material, by either light or electron microscopy, suggesting that the cell is not actively phagocytic (Williams & Williams, 1983) while the reported cases and reviews entitled GAE continue to show a combination of aggregates of leukocytes around blood vessels, findings of cysts and trophozoites, areas of haemorrhagic necrosis and fibrinoid necrotizing panarteritis (Fig. 3) and feature immunohistochemical staining of the trophozoites and granular antigens (Guarner et al., 2007; Silva et al., 2010) within the blood vessel walls and cytoplasm of macrophages of the lesions caused by protists such as Acanthamoeba and Balamuthia spp. The terminology ‘amoebic meningoencephalitis’ better suits these types of histopathological findings than the currently used classification of PAM caused by N. fowleri and GAE caused by Acanthamoeba and Balamuthia spp. (Guarner et al., 2007).

The non-specific use of the terminology of GAE has risen to alarming levels in the past decade in particular. Microscopic examination of central nervous system lesions in protist infections requires diligence and experience, and consideration of important differential diagnoses, such as primary angiitis of the central nervous system, vasculitis, giant cell arteritis and infection by viruses, fungi and other microbes, should be considered as there are no specific characteristics in clinical, laboratory or radiological findings for the diagnosis of GAE (Guarner et al., 2007). Scientific papers and original articles as well as extensive reviews and text books are mostly reporting these lesions to be granulomatous without sufficient data based on the aforesaid cytokines and/or morphological criteria. With confusion of early type IV hypersensitivity reactions with granuloma/granulomatous inflammations, there is a danger that this may obscure our understanding of the pathogenesis of these protist infections. The granulomatous immune response has attracted much interest by clinical immunologists as well as clinical practitioners. Although granulomatous inflammation is a peculiar form of chronic inflammatory response, its identification has been useful, as it narrows the differential diagnosis (Kumar et al., 2010) and at times tends to be the basis of a particular diagnosis as well. Although questions remain about the detailed understanding of the pathogenesis and cytokines that evolve this variant of tissue morphology, considerable advances have been made in our understanding of the nature of the microbial agents, homeostasis and functions of T-cells in granulomatous lesions (Unanue & Benacerraf, 1973). There is a chance that, if not contained, authors will continue to elect to quote the term GAE in AIDS patients from past published references in their original articles and reviews, which is likely to cloud the concept of this terminology and its pathogenesis.

Furthermore, as our understanding of the immunological response to protist infections has grown, so too has the apprehension that some aspects of its true pathogenesis and morphology might be overlooked if use of the term GAE does persist for immunocompromised states such as patients with AIDS or other types of T-cell-depleted immune diseases. All this can be avoided if the terminology is kept more stringent. Granulomatous inflammatory reactions should be separated from the more classically recognized cellular reactions, because of the pathological appearance of the tissue reactions, cytokines involved and the prerequisite presence (Kumar et al., 2010) of T-cell-dependent cellular immunity in the development of a granulomatous inflammatory host reaction.

Possibly the unavailability of imaging and/or diagnostic modalities and lack of in-depth understanding of the pathogenesis and morphology of emerging infections by Acanthamoeba and Balamuthia spp. and N. fowleri meningoencephalitis in the 1960s was the reason for the usage of this term in the absence of a better terminology. Because of its differential diagnostic contributions, the terminology for granulomatous inflammatory diseases, which occur in only a handful of cases, should be used judiciously.

In medical practice, physicians sometimes use the term ‘granuloma’ loosely to mean ‘a tiny nodule’. Radiologists often use the term granuloma/granulomatous when they observe a calcified nodule on X-ray or computed tomography scan of the chest and elsewhere. A few examples of these occurrences in fields other than parasitology are terms like vocal cord granuloma, pyogenic granuloma and pulmonary granulomatous used in other disciplines of medicine.

It is the privilege of journals to develop their own editorial preferences and for authors to use the nomenclature they feel best suits their study, but both these communities can join together to help contain the unjustified use of the term...
GAE in immunocompromised patients until supported by sufficient evidence, and to limit the confusion that can originate regarding the morphology and underlying mechanism of such lesions in patients with AIDS and related disorders.

A possible substitute to resolve this issue is to use the term ‘-oid’ as a suffix to describe similar lesions that do not truly fulfil the morphological and immunopathogenetic criteria of being granulomatous but that cannot be given a distinct nomenclature. Although a neologism, the term ‘granulomatoid’ might help contain the overuse of the term ‘granulomatous’ for lesions that satisfy some, if not all or most, of the cytokine and morphological criteria of GAE caused by *Acanthamoeba* and *Balamuthia* spp. and/or related pathogens in immunocompromised patients such as those with AIDS.

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**Abdul Mannan Baig**

Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan

**Correspondence**: Abdul Mannan Baig (abdul.mannan@aku.edu)

**Abbreviations**: GAE, granulomatous amoebic encephalitis; PAM, primary amoebic meningoencephalitis.


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