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inflammatory signs to be expected in some children with autoimmune-mediated encephalopathy were mainly absent, as were antibodies against OV. These two studies question whether autoimmune mechanisms have etiological significance in NS.

A recent etiologic hypothesis for NS, not addressed by Johnson and colleagues, is based on the case association with prior measles infection shown in Ugandan NS cases, and the striking clinical overlap between NS and some cases of the post-measles disorder subacute sclerosing panencephalitis (SSPE) [7]. In SSPE, masses of mutant measles nucleocapsids form intranuclear crystalline inclusions in neurons and glial cells known as Cowdry bodies. Preliminary neuropathological findings from the US Centers for Disease Control and Prevention in three Ugandan cases of NS revealed intracellular crystalline-like structures observed in histological sections of the brainstem (pons) examined by polarized light microscopy (http://www.monitor.co.ug/News/National/Nodding-disease–Crystals-found-in-victims–brains/688334-2403276-k6t33jz/index.html). While formal publication of these data is awaited, and preparation artifact should be excluded, it is noteworthy that such inclusion bodies are not seen in CNS disorders of autoimmune etiology but are reminiscent of the Cowdry bodies seen in SSPE. Infantile measles infection and ongoing malnutrition in children who develop NS – both well-known causes of immunosuppression – would open the door for heavier OV infection in NS subjects, which in turn would be consistent with higher OV-derived leiomodin-1 antibody titers in individuals with NS compared to village controls. Importantly, comparable opportunistic infections with nematodes other than OV, including *Mansonella* sp., which, like OV, is also significantly associated with South Sudan and Ugandan NS cases compared to village controls [4], are seen in untreated HIV-immunocompromised patients [10].

In summary, the findings of Johnson and colleagues [5] contribute to the neurobiology and clinical picture of NS as they demonstrate that (i) leiomodin-1 is present in human neurons, (ii) leiomodin-1 auto-antibodies cross-react with OV protein, and (iii) leiomodin-1 autoantibodies are more frequently found in cases of NS than in village controls without NS. Although these results extend the previously established association between OV infection and NS, they do not in our opinion support the authors' statement that "*This syndrome can now be added to a growing list of autoimmune epilepsies*". Further studies, including detailed neuropathological examination of well-fixed brain tissue, are warranted to determine the definitive cause of NS.

[1]Department of Neurology, School of Medicine, and Oregon Institute of Occupational Health Sciences, Oregon Health & Science University, Portland, OR, USA
[2]Department of Neurology, Medical University of Innsbruck, Innsbruck, Austria
[3]Centre for Global Health, Institute of Health and Society, University of Oslo, Oslo, Norway
[4]Centre for Global Health, Department of Neurology, Technical University of Munich, Munich, Germany

*Correspondence: spencer@ohsu.edu (P.S. Spencer).

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References

1. Iyengar, P.J. (2014) Prevalence of nodding syndrome – Uganda, 2012–2013. *Morb. Mortal. Wkly Rep.* 63, 603–606
2. Winker, A.S. (2008) The head nodding syndrome – clinical classification and possible causes. *Epilepsia* 49, 2008–2015
3. Spencer, P.S. *et al.* (2016) Nodding Syndrome: 2015 International Conference Report and Gulu Accord. *eNeurologicalSci* 3, 80–83
4. Dowell, S.F. *et al.* (2013) Nodding syndrome. *Emerg. Infect. Dis.* 19, 1374–1384
5. Johnson, T.P. *et al.* (2017) Nodding syndrome may be an autoimmune reaction to the parasitic worm *Onchocerca volvulus*. *Sci. Transl. Med.* 9, eaaf6953
6. Winkler, A.S. *et al.* (2014) A longitudinal study on nodding syndrome – a new African epilepsy disorder. *Epilepsia* 55, 86–93
7. Spencer, P.S. *et al.* (2016) Environmental, dietary and case-control study of Nodding Syndrome in Uganda: a post-measles brain disorder triggered by malnutrition? *J. Neurol. Sci.* 369, 191–203
8. Soldatos, A. *et al.* (2015) Evaluation and immunomodulatory treatment at the NIH of children with nodding syndrome from Northern Uganda. *Neurology* 84 (Suppl), S37.005
9. König, R. *et al.* (2010) The role of *Onchocerca volvulus* in the development of epilepsy in a rural area of Tanzania. *Parasitology* 137, 1559–1568
10. Brown, M. *et al.* (2004) Helminth infection is not associated with faster progression of HIV disease in coinfected adults in Uganda. *J. Infect. Dis.* 190, 1869–1879

Forum

Malaria Eradication and the Hidden Parasite Reservoir

Miles B. Markus[1,2,*]

Accumulation of erythrocytic parasites in bone marrow and the spleen has been reported in cases of *Plasmodium vivax* malaria. If this occurs commonly, these stages represent a possible source of early, relapse-like homologous recurrences. Moreover, they might hinder the elimination of malaria from human populations. Pertinent research suggestions have been made.

Plasmodium vivax Relapse: Beyond the Hypnozoite

Ever since the first half of the last century, a reiterated comment that artificially induced *Plasmodium vivax* malaria [1] does not relapse (Box 1) following injection of erythrocytic stages (versus sporozoite inoculation) has occasionally appeared in the literature. Treatment is conventionally given relatively promptly upon detection of bloodstream parasites [1], and this repeated remark is based on the post-therapy course of infections initiated from both sources. It is certainly true that adequately treated, blood-stage-induced *P. vivax* malaria does not recur (Box 1), as far as is known. Sporozoite-transmitted infections, on the other hand, frequently do recur. This is sometimes adduced as support for the hypnozoite hypothesis of relapse, and probably correctly so. What remains to be determined, however, is whether or not hypnozoites are the only source of relapse-like recurrence of naturally acquired *P. vivax* malaria. That is an open question, discussed here in the practical context of malaria eradication.

Box 1. Terms for Recurrent Malaria

The definition of the word 'relapse' in relation to malaria is somewhat arbitrary. Nowadays, the meaning differs from older, outdated interpretations. Modern malariologists generally use 'relapse', which is synonymous with 'true relapse', when referring to renewed bloodstream parasitemia that is presumed to be hypnozoite-initiated, as well as to presumed hypnozite-mediated reappearance of clinical illness. By contrast, a 'recurrence' may be the result of a relapse, a recrudescence or reinfection. Whereas 'recurrence' covers all three of these possibilities, 'recrudescence' refers exclusively to a recurrence that originates from a parasite(s) belonging to the same population that gave rise to the primary infection.

Potential 'New' Origin of Relapse-like Malarial Recurrence

The life cycle of *P. vivax* is shrouded in mystery. One question that arises is whether or not treatment so early (see above) of both blood-stage-induced and sporozoite-initiated infections nips in the bud the accumulation of erythrocytic parasites that may otherwise have taken place in bone marrow and perhaps also in the spleen and other sites [2]. In someone naturally infected but semi-immune and asymptomatic, there could be considerable opportunity for blood-stage parasites to accumulate, if this normally occurs and to any significant extent. Future research should shed light on the situation. These parasites might usually, but not necessarily always, be completely cleared in symptomatic malaria, depending on the drug(s) used.

Because induced human *P. vivax* malaria is usually treated early on, much more information is available for untreated experimental *Plasmodium cynomolgi* malaria in monkeys. A variable number of recurrences may take place in *P. cynomolgi* infections. These recurrences can follow irrespective of whether the invading organisms were sporozoites or subinoculated erythrocytic forms [3,4]. The fact that blood-stage-initiated, untreated, simian *P. cynomolgi* malaria can recur is of interest because this host–parasite combination is an accepted model for *P. vivax* malaria in humans [5].

Recrudescent (Box 1) bloodstream malaria parasites are genetically homologous to the bloodstream parasites from which they are clonally descended. Recrudescences originate from merozoites. By contrast, a relapse is nowadays defined as being hypnozoite-mediated (Box 1), and hypnozoites are thought to be directly sporozoite-derived, following entry of sporozoites into the primate host (Box 2). When reinfection is excluded, how should homology be interpreted in naturally acquired, recurrent *P. vivax* or *P. cynomolgi* malaria and in sporozoite-initiated experimental disease? I do not believe that, in the current state of knowledge, it can confidently be concluded (if known that reinfection has not taken place) that a given homologous recurrence is a hypnozoite-associated relapse, not even when it is a long-term homologous recurrence (Box 2). The cause could be blood stages. Although an activated hypnozoite [6] can theoretically be homologous, let us assume for purposes of speculation that a hypnozoite is not the origin of a particular homologous *P. vivax* or *P. cynomolgi* recurrence. What could alternatively give rise to that recurrence? Failure of a schizontocide to kill all the parasites in the bloodstream is the classical presumed situation in which a recrudescent recurrence takes place. To give another example of a potential parasite source, it has been suggested that in mosquito-transmitted *P. vivax* malaria, noncirculating erythrocytic forms can possibly be prevalent in bone marrow and perhaps also in the spleen. However, the extent to which the occurrence of blood stages in bone marrow and the spleen is frequent or important is not known [2]. Erythrocytic *P. vivax* parasites could conceivably accumulate elsewhere as well. In epidemiological surveys that inform malaria elimination, can blood stages be present in, for example, bone marrow, despite a negative blood PCR result?

P. vivax has a marked predilection for reticulocytes, but parasitizes normocytes. If *P. vivax* does regularly occur outside the peripheral circulation in, for example, bone marrow, for how long can parasites in normocytes remain there (the lifespan of normocytes is approximately 4 months)? Are they able to persist intracellularly for any length of time without actively multiplying and causing the cell to rupture? Can erythrocytic plasmodial stages in bone marrow and/or the spleen be a source of at least some early, homologous, nonhypnozoite-mediated recurrences of *P. vivax* malaria, resulting in or contributing to parasite repopulation of the peripheral blood to a transmissible level? I suggest that this is possible. Microscopically detectable recrudescence could easily follow

Box 2. Future Research Possibilities

- Genetic studies might help to ascertain whether relapse-like malarial recurrence is ever caused, via erythrocytic schizogony, by any noncirculating, nonhypnozoite tissue stage(s). In sporozoite-induced *Plasmodium cynomolgi* infection, parasites from blood drawn during early and late recurrences, respectively, should be compared with those responsible for the initial parasitemia. Heterologous *P. cynomolgi* recurrences would be compatible with the hypnozoite theory of relapse, because the (single) sporozoite inoculum will have been genotypically diverse (applicable to long-established laboratory isolates too) and hypnozoites appear to be directly sporozoite-derived. If homologous recurrences are detected, it should not be assumed that they (including long-term ones) are all hypnozoite-mediated. Whether at least some putative, homologous *P. cynomolgi* recurrences can be recrudescences arising from noncirculating parasites such as erythrocytic forms in bone marrow or the spleen (these should be searched for) is an open question.
- To quote [12]: 'The idea that the bone marrow is a cryptic site for the life cycle of *P. vivax* should be investigated ...'.
- The distribution of *P. vivax* in extrahepatic tissues of susceptible nonhuman primates should, if possible, be researched in order to better understand the nature of the nonhepatic parasite reservoir in relation to the attempted eradication of malaria.

renewed or increased asexual reproduction of parasites that might take place, immunity permitting, inside red blood cells in the bone marrow or spleen, as it does when schizogony occurs in the bloodstream.

Extrapolation from *Plasmodium malariae* Infection

Following on from the previous section, can facts for *P. malariae* provide any clues as to whether some homologous *P. vivax* and *P. cynomolgi* recurrences might be hypnozoite-independent? Whether or not this is like comparing apples with pears is unknown. That aside, *P. malariae* infection is long-lasting after injection of blood stages, as after inoculation with sporozoites [3]. Even if (as for *P. vivax* and *P. cynomolgi*) there is a hypnozoite form in the life cycle of *P. malariae* (something that is uncertain [7,8]), not only short-term but also the long-term recurrences for which *P. malariae* is well known cannot, in blood-stage-induced infections, be relapses. This is because, as explained earlier, hypnozoites appear to be obligatorily sporozoite-derived (Box 2); and post-passage, blood-stage *P. malariae* inocula do not contain sporozoites. Such *P. malariae* recurrences must therefore be recrudescences that originate from quiescent merozoites.

By analogy, is sporozoite-acquired *P. vivax* or *P. cynomolgi* malaria (in addition to exhibiting hypnozoite-associated relapses) able to recrudesce, just as blood-stage-initiated *P. malariae* infection does? In other words, can *P. vivax* and *P. cynomolgi* recurrences originate from erythrocytic parasites in bone marrow, for instance? If so, that would be a non-hypnozoite explanation for an unknown percentage of the homologous recurrences that have been encountered by researchers when studying naturally contracted *P. vivax* malaria.

Whereabouts do persisting *P. cynomolgi* and *P. malariae* forms occur in blood-stage-induced chronic malaria [3,4] and, other than in the liver, in sporozoite-transmitted disease? *P. malariae* infection can be caused by blood transfusion. Accordingly, *P. malariae* must be present at least periodically in the peripheral blood. Parasitemia is presumably controlled by immunity [9], the specific trigger(s) for reactivation being unknown. *P. vivax*, *P. cynomolgi*, and other mammalian plasmodial species in the bloodstream are not always seen only in erythrocytes [4,10,11]. Similar dual tropism might thus apply to *P. malariae*, which has been known to survive in the body for more than 50 years (and *Plasmodium falciparum*, with unknown frequency, for longer than 10 years). Does accumulation of *P. cynomolgi* and *P. malariae* take place, as *P. vivax* might to whatever extent [2], in bone marrow and perhaps the spleen (Box 2)? For *P. cynomolgi* to be of use as a model organism in this particular respect, the overall pattern of accumulation or nonaccumulation would need to resemble that of *P. vivax* (which it might well do).

Concluding Remarks and Future Perspectives

It is generally recognized that *P. vivax* hypnozoites, which reside in the liver, are a presumed obstacle to success in eradicating malaria. However, so could nonhypnozoite forms that might persist elsewhere in the body, such as blood-stage parasites in bone marrow and splenic tissue. Wherever erythrocytic schizogony occurs and leads to renewed or increased peripheral parasitemia, formation of gametocytes and their ingestion by mosquitoes could follow. The consequence will be ongoing transmission of malaria.

Thus, an outstanding question in relation to the hidden parasite reservoir is whether recurrences in sporozoite-transmitted *P. vivax*, *P. cynomolgi* and certain other nonhuman primate malarial infections can have a bimodal origin, resulting in either recrudescence or relapse. For instance, could putative homologous *P. cynomolgi* recurrences in sporozoite-infected monkeys often be recrudescences rather than relapses, analogous in source to recrudescences that follow inoculation of erythrocytic *P. cynomolgi* organisms [3,4]? If this does happen, then how frequently does 'pseudorelapse' take place, versus hypnozoite-initiated relapse? *Inter alia*, genotyping using the *P. cynomolgi* model for *P. vivax* malaria (Box 2) might prove to be informative in elucidating the matter of the hidden parasite reservoir and facilitating malaria elimination. At present, there are more questions than answers.

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Any finding, conclusion, recommendation, or opinion expressed here is that of the author; and the NRF does not accept any responsibility in this regard.

[1]School of Animal, Plant, and Environmental Sciences, Faculty of Science, University of Witwatersrand, Johannesburg, South Africa
[2]Wits Research Institute for Malaria, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

*Correspondence: medsynth@yahoo.co.uk (M.B. Markus).

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References

1. Payne, R.O. *et al.* (2017) *Plasmodium vivax* controlled human malaria infection – progress and prospects. *Trends Parasitol.* 33, 141–150
2. Barber, B.E. *et al.* (2015) Parasite biomass-related inflammation, endothelial activation, microvascular dysfunction and disease severity in vivax malaria. *PLoS Pathog.* 11, e1004558
3. Corradetti, A. and Verolini, F. (1950) Studies on relapses in blood-induced infections from *Plasmodium malariae* and *Plasmodium cynomolgi*. *J. Natl. Malar. Soc.* 9, 327–331
4. Schmidt, L.H. *et al.* (1982) I. The characteristics of untreated sporozoite-induced and trophozoite-induced infections. *Am. J. Trop. Med. Hyg.* 31, 612–645
5. Waters, A.P. *et al.* (1993) Evolutionary relatedness of some primate models of *Plasmodium*. *Mol. Biol. Evol.* 10, 914–923
6. Mikolajczak, S.A. *et al.* (2015) *Plasmodium vivax* liver stage development and hypnozoite persistence in human liver-chimeric mice. *Cell Host Microbe* 17, 526–535
7. Richter, J. *et al.* (2016) Clinical implications of a gradual dormancy concept in malaria. *Parasitol. Res.* 115, 2139–2148

8. Sutherland, C.J. (2016) Persistent parasitism: the adaptive biology of malariae and ovale malaria. *Trends Parasitol.* 32, 808–819
9. Vinetz, J.M. *et al.* (1998) *Plasmodium malariae* infection in an asymptomatic 74-year-old Greek woman. *N. Engl. J. Med.* 338, 367–371
10. Blanc, F. and Cros, R. (1952) Note sur la présence d'un schizonte dans un monocyte du sang circulant. *Méd. Trop.* 12, 83–84
11. Landau, I. *et al.* (1999) Survival of rodent malaria merozoites in the lymphatic network: potential role in chronicity of the infection. *Parasite* 6, 311–322
12. Russell, B.M. and Cooke, B.M. (2017) The rheopathobiology of *Plasmodium vivax* and other important primate malaria parasites. *Trends Parasitol.* 33, 321–334

Forum

Salivary Prostaglandin E2: Role in Tick-Induced Allergy to Red Meat

Alejandro Cabezas-Cruz,[1,2] Lourdes Mateos-Hernández,[3] Jindřich Chmelař,[2] Margarita Villar,[3] and José de la Fuente[3,4,*]

Tick-induced allergy to red meat is associated with anti-α-Gal IgE antibody levels. We propose that tick salivary prostaglandin E2 triggers antibody class switching in mature B cells, increasing the levels of anti-α-Gal IgE antibodies. Immune tolerance to α-Gal in blood type B individuals might reduce the risk to this allergy.

Tick-induced allergy to red meat is becoming a global problem with increasing prevalence in the USA, Australia, and Europe, and several tick species have been implicated in these disorders [1]. Remarkably, most of the patients that become allergic, had tolerated red meat for many years before being sensitized by tick bites [1]. This finding suggests that anti-Galα1-3Galβ1-(3)4GlcNAc-R (α-Gal) IgE antibodies induced by tick bites, break the oral tolerance to food allergens. This tick-induced immune response is antigen-specific and results in gut-related but not lung-related allergy.

Tick saliva is a complex mixture of pharmacologically active compounds. Tick saliva and/or tick salivary gland extracts were shown to inhibit almost every defensive mechanism and affect leukocyte populations through immunomodulatory, antihemostatic and anti-inflammatory molecules [2]. Transcriptomics studies of tick salivary glands discovered clusters of related proteins that are referred to as multigene families and usually contain tens or even hundreds of more or less similar proteins, with protease inhibitors being the most abundant group of tick salivary secreted proteins in *Ixodes scapularis* [2]. Interestingly, the genes coding for these proteins are usually expressed sequentially throughout tick feeding, bringing up the question of whether this phenomenon could be a form of antigenic variation [2].

Apart from proteins with immunomodulatory activity, ticks also produce nonprotein molecules such as prostaglandin E2 (PGE_2), which is synthetized in the tick salivary glands and secreted via the saliva into the feeding lesion [3]. Several tick species from major genera such as *Amblyomma*, *Ixodes*, and *Rhipicephalus*, which have been involved in tick-induced allergies, were found to secrete PGE_2 in their saliva [3,4]. Tick salivary PGE_2 was reported to have an immunomodulatory effect [3,4]. In particular, PGE_2 inhibited cytokine production by inducing cyclic AMP-proteins kinase A (cAMP-PKA) signaling in dendritic cells [3]. While attention has been paid to the immunomodulatory effect of tick salivary PGE_2 on dendritic cells [3,4], the effect of PGE_2 on B cells

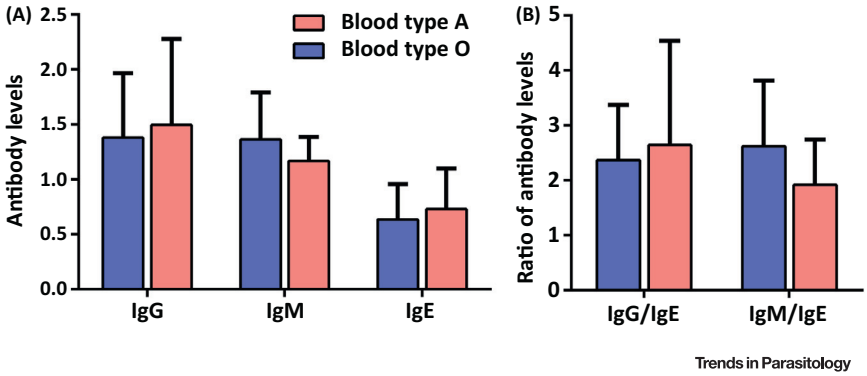

Figure 1. ABO Blood Types and Antibody Response to α-Gal: A Case Report. The ABO blood types are important self-antigens with implications in immune tolerance and xenotransplantation [6]. Humans have antibodies against missing A/B blood type antigens. As shown in a series of studies, the levels of anti-α-Gal antibodies are lower in individuals with blood type B [8]. This finding may be related to the fact that monoclonal and polyclonal anti-α-Gal antibodies show strong interaction with both galactose residues of α-Gal [11]. Therefore, the presence of galactose residues in blood type B antigen may be sufficient for the binding of anti-α-Gal autoantibodies to blood type B antigen, resulting in total or partial tolerance to Gal–Gal blocks in individuals with blood type B. The figure shows the analysis of anti-α-Gal IgG, IgM, and IgE ratios in healthy individuals with blood types O and A, which should have similar levels of anti-α-Gal antibodies. The dataset containing the anti-α-Gal antibody levels in healthy adults from the Iberian Peninsula was published by Cabezas-Cruz *et al.* [12]. (A) Anti-α-Gal IgG, IgM and IgE antibody levels (O.D. 450 nm) were determined by ELISA in sera from healthy individuals [12]. Anti-α-Gal IgE levels are lower than anti-α-Gal IgG and IgM levels in healthy individuals with blood types A and O. (B) Anti-α-Gal IgG/IgM/IgE ratio analysis shows that no significant differences ($P > 0.05$) exist between IgG/IgE and IgM/IgE ratios in healthy individuals with blood type A or O. These results support our hypothesis that tick bites may induce class switch recombination (CSR), which will result in an increase in anti-α-Gal IgE in individuals with blood types A and O. Subsequently, IgG/IgE and IgM/IgE ratios are expected to decrease below the values shown in the Figure (i.e., for blood type O individuals, IgG/IgE<2.36 with SD ± 1.0 and IgM/IgE<2.61 and SD ± 1.2, and for blood type A individuals, IgG/IgE<2.64 and SD ± 1.9 and IgM/IgE<1.92 and SD ± 0.8).