Altered brain bioenergetics in murine cerebral malaria depend on infection susceptibility and cytokine knockout status

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Introduction Malaria infection can cause cerebral symptoms without parasite invasion of brain tissue. Biochemical changes include increased inorganic phosphate (Pi) and decreased PCr, and increased PME, consistent with unfavourable bioenergetic status and infection, respectively [1]. This is also associated with changes to metabolism, including increased glutamatergic activity, and increased expression of cytokines in the brain, particularly interferon-γ (IFNγ), TNF-α and lymphotoxin-α (LTα). In this work we examined the bioenergetic response of the brain in cytokine knockout mice infected with cerebral malaria.

Methods Mice (C57BL/6 (ctl) or knock-outs of) were inoculated by intraperitoneal injection of 10^6 red blood cells infected with *P. berghei* ANKA (PbA) or 100 µl saline (control). In addition mice deficient (-/-) in IFNγ, TNF-α and LTα, produced on a C57BL/6 background were similarly treated. On day 6-7 post infection mice were anaesthetised i.p. with Nembutal. ^{31}P spectra were obtained at 9.4T using a ^{1}H/^{31}P-tuned surface coil (T_R = 2s, NS = 400). Spectra were processed using jMRUI (Versions 1.3 or 2.1). AMARES was used to fit Lorentzian lineshapes to resonance frequencies assigned to PME, inorganic phosphate, PDE, phosphocreatine and α,β and γ ATP. Individual metabolites were expressed as a percentage of the total signal.

Results There were no significant differences in the ^{31}P spectra from the brains of any uninfected mouse group studied. C57BL/6 mice infected with PbA showed similar ^{31}P changes to those reported in CBA mice [1]. IFNγ and LTα KO mice had ^{31}P spectra which were not significantly different to those from control uninfected mice, while TNF-α mice showed increased Pi and decreased PCr peaks, similar to those seen in infected mice (Fig. 1). TNF-α mice, however, did not show the significant increase in the PME resonance seen in infected controls.

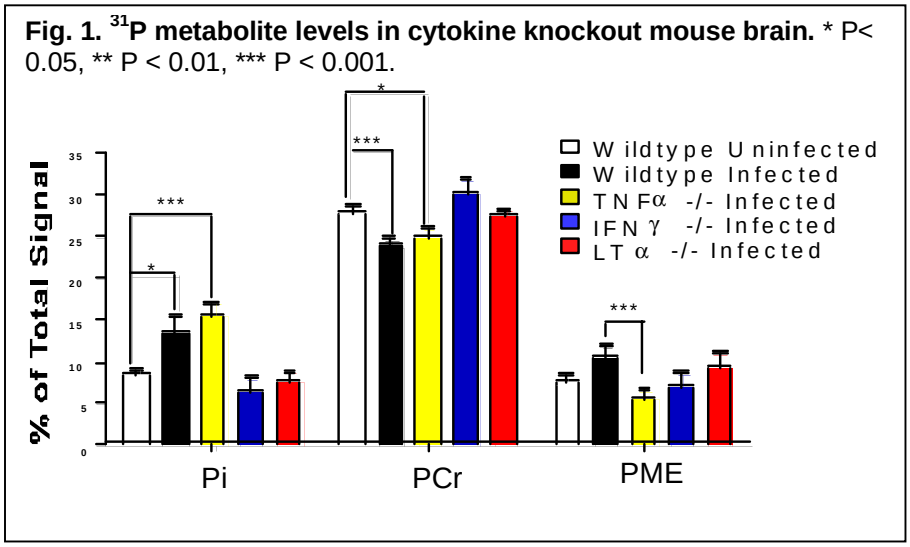

Fig. 1. ^{31}P metabolite levels in cytokine knockout mouse brain. * P< 0.05, ** P < 0.01, *** P < 0.001.

Discussion Infected control mouse brains showed changes consistent with poorer bioenergetics. While ATP levels were not significantly altered, the total amount of PCr decreased and Pi increased, consistent with alteration in bioenergetic steady-state equilibria. IFNγ and LTα KO mice are resistant to cerebral infection with PbA [2, 3] and showed no significant ^{31}P MRS changes compared to uninfected control, while TNF-α KO mice, which are susceptible, showed similar Pi and PCr changes to infected controls. The increase in PME seen in infected controls was absent in TNF-α KO mice suggesting a role for this cytokine in mediating this change. These findings support the suggestion that the ^{31}P MRS changes seen in cerebral malaria infection are related to susceptibility to the disorder and are consistent with the cytopathic hypoxia theory of cerebral malaria [4]

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