

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Biochimica et Biophysica Acta 1637 (2003) 183–186

[www.bba-direct.com](http://www.bba-direct.com)

Rapid report

## Elevated anticardiolipin antibodies in acute liver failure

K.R. Rozee<sup>a</sup>, P. Acott<sup>b,c</sup>, S.H.S Lee<sup>a</sup>, J.F.S. Crocker<sup>b,c</sup>, D. Gough<sup>a</sup>, J. MacDonald<sup>a</sup>,  
J. Evans<sup>d</sup>, M.G. Murphy<sup>d,\*</sup><sup>a</sup>Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7<sup>b</sup>Department of Pediatrics, Dalhousie University Halifax, Nova Scotia, Canada B3J 3G9<sup>c</sup>IWK Health Centre, Halifax, Nova Scotia, Canada B3J 3G9<sup>d</sup>Department of Physiology and Biophysics, Dalhousie University, Sir Charles Tupper Medical Building, Halifax, Nova Scotia, Canada B3H 4H7

Received 30 December 2002; received in revised form 4 February 2003; accepted 27 February 2003

**Abstract**

Antibodies to cardiolipin (aCLA), a phospholipid primarily localized in inner mitochondrial membranes, were transiently elevated ( $P < 0.01$ ) when mice were exposed to an industrial surfactant and then infected with influenza B virus, a model of acute liver failure (ALF). Children with ALF also had elevated levels of aCLA.

© 2003 Elsevier Science B.V. All rights reserved.

**Keywords:** Anticardiolipin antibody; Acute liver failure; Reye's syndrome; Surfactant/influenza B mouse model

Cardiolipin (diphosphatidylglycerol), a very minor component of most mammalian membranes, but a major component of inner mitochondrial membranes [1], initiates an antibody response in a variety of diseases involving mitochondrial damage. It has long been known that patients with autoimmune diseases (e.g., systemic lupus erythematosus [SLE], rheumatoid arthritis) and thromboembolic diseases (e.g., stroke, myocardial infarction) regularly produce high levels of anticardiolipin antibodies (aCLA) [2]. However, aCLA have also been shown to be elevated in the absence of systemic disease, in patients taking various drugs (e.g., valproate, phenytoin) or with a spectrum of viral infections (e.g., hepatitis C, varicella zoster) [2,3]. Increases in aCLA have also been observed in SLE patients following vaccination with inactivated influenza [4] and in patients with Sjögren's syndrome following infection with influenza A [5].

Acute liver failure (ALF), one example of which is Reye's syndrome (RS), appears to be an injury cascade that leads to derangement of hepatic mitochondrial membranes and organelle dysfunction [6]. It is a heterogeneous syndrome that may result from an interaction between viruses such as influenza B (FluB) and one or more xenobiotic

chemicals including aspirin, valproic acid and industrial surfactants [7]. The mechanism(s) underlying ALF have not been identified, although energy metabolism appears to be severely compromised both in humans and in animal models [7]. As we have demonstrated that there is extensive mitochondrial damage in our surfactant/FluB mouse model of ALF (Fig. 1), we proposed that aCLA would be elevated in this model. To test this hypothesis, we analyzed aCLA levels in sera from mice at various times throughout the in vivo experiment. As well, archived sera from five children with symptoms consistent with ALF, and six non-ALF patients of similar ages were analyzed for aCLA.

The surfactant/FluB mouse model, which reproduces many of the features of human ALF, has been described previously [7]. Briefly, neonatal mice were dermally exposed to either minimal essential media (MEM, vehicle control) or a dilute solution of the industrial surfactant, Toximul (Tox), daily for 12 days. On postnatal (P) day 13 (P13), half of each group were lightly anaesthetized with ether and inoculated with a sublethal dose ( $LD_{10-20}$ ) of mouse-adapted FluB. The remainder were anaesthetized only. Deaths in the four experimental groups (MEM, Tox, FluB, Tox + FluB) were recorded daily until P21 when remaining animals were killed. At the times of sacrifice, bloods were collected and sera were separated and stored at  $-86^{\circ}\text{C}$  until analysis. aCLA were analyzed using two methods. The first was an in-house, solid phase enzyme

\* Corresponding author. Tel.: +1-902-494-3713; fax: +1-902-494-1685.

E-mail address: [mary.murphy@dal.ca](mailto:mary.murphy@dal.ca) (M.G. Murphy).

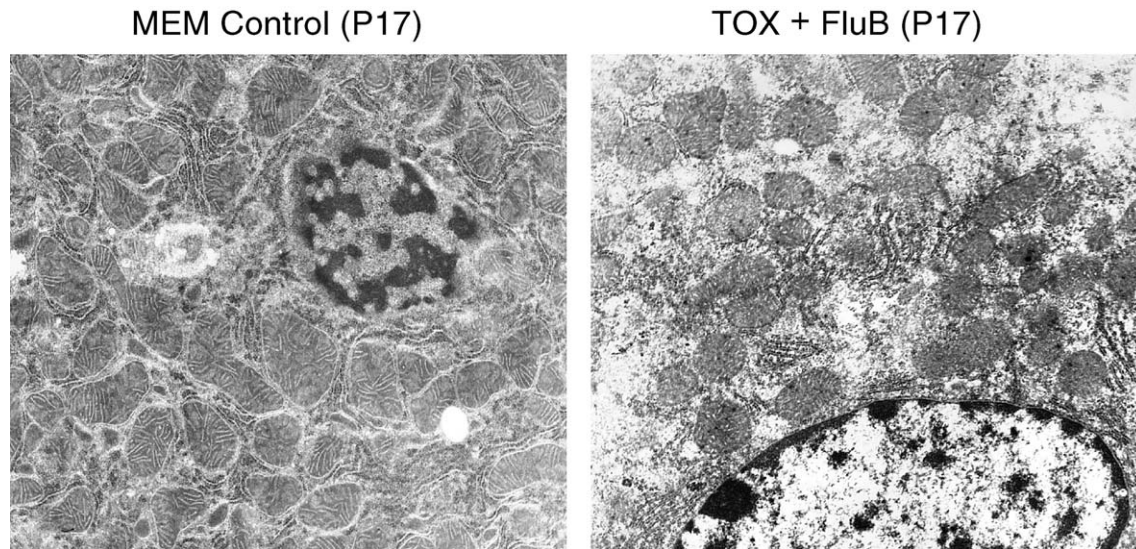


Fig. 1. Electron microscopic assessment (17,000 $\times$  magnification) of livers from control (MEM) and Tox + FluB-treated mice on experimental day 17 (P17). Mice were exposed to Tox and subsequently infected with FluB as described in the text. The livers of the treated mice showed prominent cytoplasm of cellular swelling, disorganization of the endoplasmic reticulum, and distorted mitochondria with poorly defined cristae.

immunoassay adapted from Koike et al. [8]. Briefly, 96-well culture plates were coated with cardiolipin (45  $\mu\text{g}/\text{ml}$  in ethanol), the solvent was dried under  $\text{N}_2$ , and the wells were washed with 0.3% gelatin in phosphate-buffered saline, pH 7.2 (gel-PBS). Sera (50  $\mu\text{l}$ ) were added to the wells and the plates were incubated at 37  $^\circ\text{C}$  for 90 min. After the wells were washed (gel-PBS followed by 1% bovine serum albumin in gel-PBS), goat anti-mouse or anti-human IgG tagged with alkaline phosphatase (AP) was added for 90 min. The plates were washed and AP substrate (*p*-nitrophenyl phosphate disodium, 100  $\mu\text{l}$ , Sigma-Aldrich) was added to each well. After 30 min, the reactions were stopped with 50  $\mu\text{l}$  of 20 nM cysteine. Optical densities were read at 410/490 nm on a microplate reader (DYNEX Technologies Inc., Chantilly, VA). The second method was done using an aCLA ELISA kit from Alpha Diagnostic International (San Antonio, TX). The data are expressed as the ratio of optical densities in test wells relative to control wells without serum. Values for the different experimental groups of mice are presented relative to standardized MEM controls (= 1.0). Statistical analysis was done using a Student's unpaired *t*-test with Bonferroni correction for multiple testing.

The results of the mouse sera analyses are illustrated in Fig. 2. Two days following FluB inoculation (P15) and before any mice had died (Fig. 2A), levels of aCLA in mice treated with Tox alone were elevated by  $\approx 20\%$ , an effect that persisted until P17, 5 days after painting had ceased (Fig. 2B). The aCLA-elevating effect of Tox had waned by P21. In mice only infected with FluB, aCLA levels were increased also by  $\approx 20\%$ ; however, the effect was not observed until P17, and persisted until at least 8 days after inoculation (P21). The most noteworthy finding was that on P17, aCLA levels in mice treated with both Tox and FluB were  $>100\%$  higher than values for the MEM group

( $P < 0.005$ ) and significantly higher than values for either the Tox alone ( $P < 0.002$ ) or FluB alone ( $P < 0.02$ ) groups. This effect coincided with the onset of mortality (Fig. 2A) and was transient in nature. Values for aCLA in the Tox + FluB group were equivalent to those for Tox alone on P15, and to those for FluB alone on P21. The mean value ( $\pm$  S.E.) for aCLA in archived sera from children diagnosed with ALF (three RS, one medium chain acyl-CoA dehydrogenase deficiency, one toxic encephalopathy) was  $>250\%$  higher than in non-ALF patients of similar ages ( $P = 0.03$ ) (Fig. 3).

Infection-induced increases in aCLA expression, in contrast to patterns seen in autoimmune diseases, tend to be relatively minor and transient [2,3,9]. This may be related to the fact that infection-related aCLA bind directly to cardiolipin, while those associated with autoimmune diseases bind tightly to a cofactor protein [1,2,10]. The clinical significance of aCLA in virus and drug settings is not known. While the elevations in autoimmune disease [2,9] and in some viral infections [3] are associated with increased risk of thrombosis, those in other diseases are believed to be nonspecific reflections of liver damage [10]. Consistent with the latter, the ALF patients whose sera had elevated aCLA (Fig. 3) had no history of thrombosis, yet had evidence of hepatic damage.

Our studies have shown that mice exposed to the presumed nontoxic industrial surfactant, Tox, and then infected with low doses of FluB have marked degeneration of hepatic mitochondria (Fig. 1) and significant transient increases in ammonia production [11]. In the present study, we observed that without Tox exposure, there were modest ( $\approx 20\%$ ) elevations in aCLA 4 days after the mice were inoculated with the respiratory FluB virus. While the lung is a presumed site of the cardiolipin stimulus, other sites are

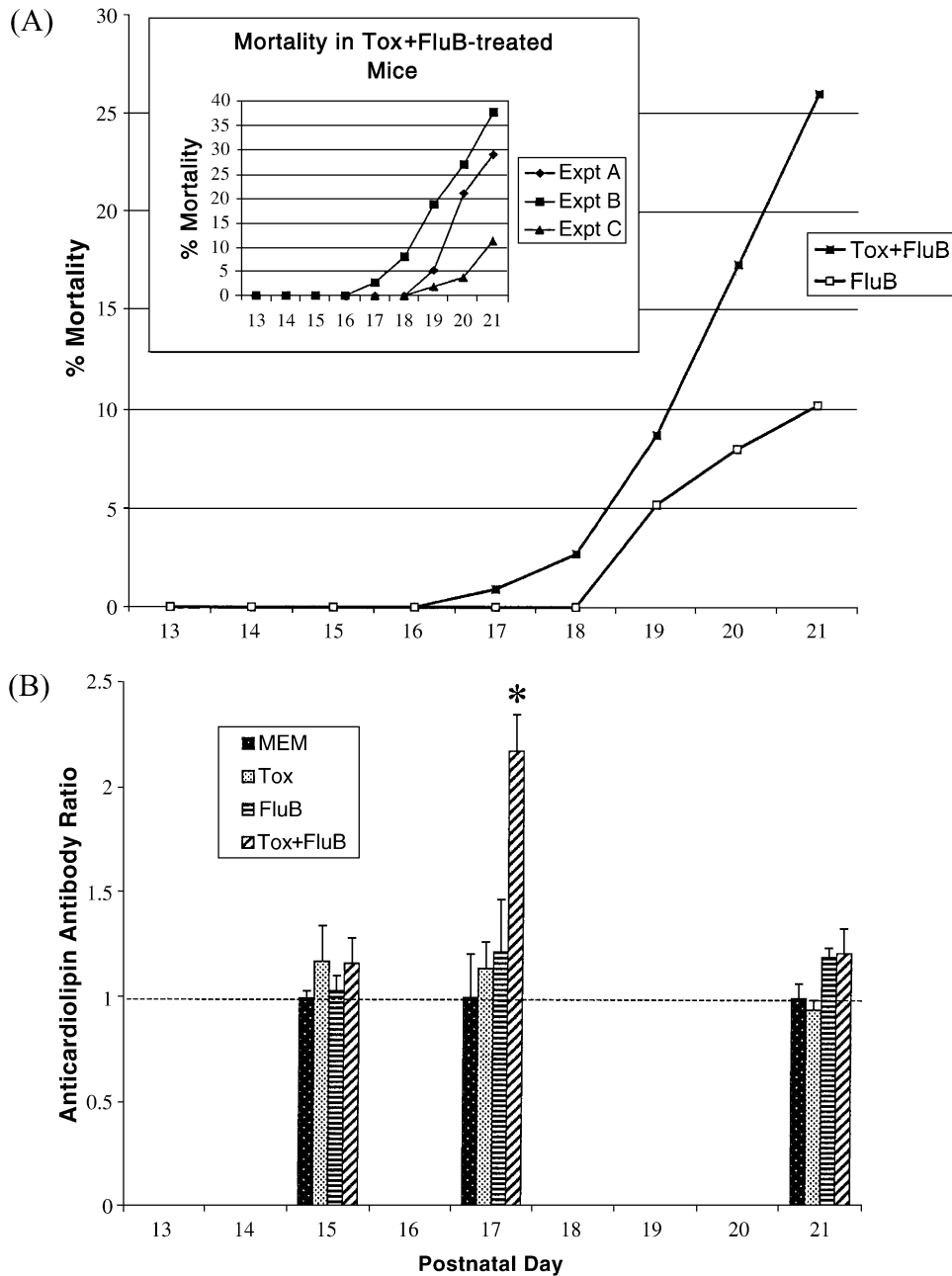


Fig. 2. Mortality and aCLA levels in the Tox + FluB mouse model of ALF. Mice were treated with MEM (control), Tox, FluB or Tox + FluB as described in the text. (A) Mean numbers of deaths that occurred up to P21 in mice only infected with FluB and in mice exposed to Tox and then infected with FluB in three separate in vivo experiments. Insert: Time courses of deaths in the Tox + FluB group in individual experiments. (B) Sera were collected at P15, P17 and P21 and were analyzed for aCLA as described in the text. Data are the means  $\pm$  S.E. of standardized values from 4 to 12 mice in each group on each sampling day. \*Data were significantly higher than all other experimental groups ( $P < 0.01$ ) on P17.

possible, as FluB antigens have been detected in human muscle [12], in brains of children with influenza A- and B-associated encephalopathy [13], and in livers of experimental animals [14,15]. This study showed that exposing young mice to Tox and then infecting them with FluB increased aCLA levels by 100% (Fig. 2B). A Tox-alone effect, although modest, preceded this interactive reaction, suggesting that surfactant exposure was an obligate partner. Although the significance of the synergistic, Tox + FluB-

mediated elevation in aCLA is not known, it is consistent with our previous demonstration that energy metabolism is severely compromised in this model [7]. Cardiolipin plays an intimate, essential role in many aspects of mitochondrial energy production [1], including carnitine-dependent transport of fatty acids across the mitochondrial membrane. Our earlier studies demonstrated that the modest inhibitions of fatty-acid  $\beta$ -oxidation with Tox or FluB treatments individually were potentiated when the

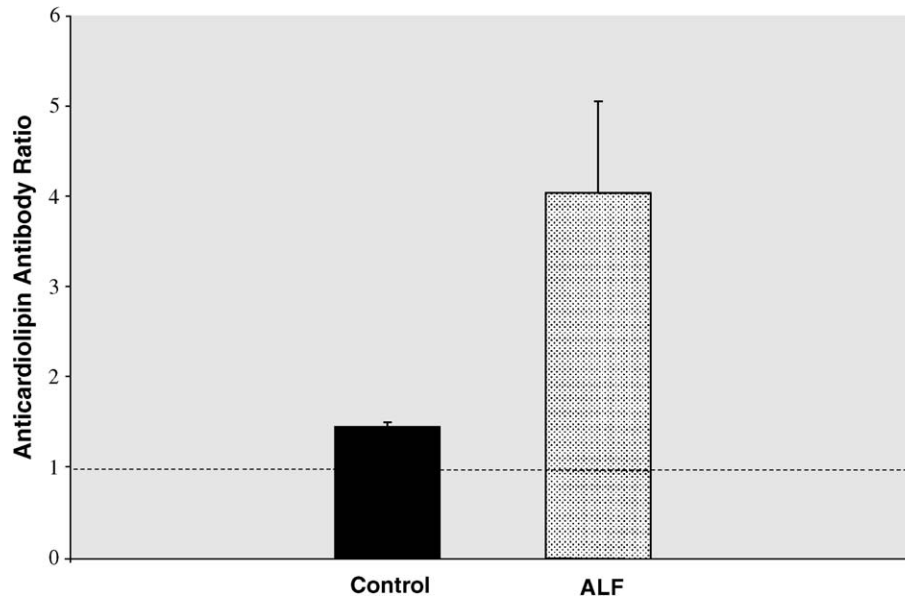


Fig. 3. aCLA levels in archived sera from children diagnosed with ALF and from age-matched controls. Sera were analyzed for aCLA as described in the text.

treatments were combined [7]. We had presumed that inhibition of  $\beta$ -oxidation was due solely to blockade of mitochondrial matrix events. However, the disruptions in mitochondrial membrane integrity suggested by the demonstrated elevations in circulating aCLA in this study lend support to the possibility that transport of fatty acids into the mitochondria is also compromised.

## References

- [1] M. Schlame, D. Rua, M.L. Greenberg, The biosynthesis and functional role of cardiolipin, *Prog. Lipid Res.* 39 (2000) 257–288.
- [2] S.C. Keswani, N. Chauhan, Antiphospholipid syndrome, *J. R. Soc. Med.* 95 (2002) 336–342.
- [3] I.W. Uthman, A.E. Gharavi, Viral infections and antiphospholipid antibodies, *Semin. Arthritis Rheum.* 31 (2002) 256–263.
- [4] M. Abu-Shakra, J. Press, S. Sukenik, D. Buskila, Influenza virus vaccination of patients with SLE: effects on generation of autoantibodies, *Clin. Rheumatol.* 21 (2002) 369–372.
- [5] E. Ulvestad, A. Kaneström, P. Tengner, S. Gjerde, J. Sundal, H.-J. Haga, Anti-cardiolipin autoantibodies and pulmonary embolism, *Scand. J. Rheumatol.* 29 (2000) 330–333.
- [6] M. Visentin, M. Salmona, M.T. Tacconi, Reye's and Reye-like syndromes, drug-related diseases? (causative agents, etiology, pathogenesis, and therapeutic approaches), *Drug Metab. Rev.* 27 (1995) 512–539.
- [7] M.G. Murphy, J.F.S. Crocker, S.H.S. Lee, P. Acott, H. Her, Sequestration of coenzyme A by the industrial surfactant, Toximul MP8: a possible role in the inhibition of fatty-acid  $\beta$ -oxidation in a surfactant/influenza B virus mouse model for acute hepatic encephalopathy, *Biochim. Biophys. Acta* 1361 (1997) 103–113.
- [8] T. Koike, M. Sueishi, H. Funaki, H. Tomioka, S. Yoshida, Anti-phospholipid antibodies and biological false positive serological test for syphilis in patients with system lupus erythematosus, *Clin. Exp. Immunol.* 56 (1984) 193–199.
- [9] A. Martini, A. Ravelli, The clinical significance of antiphospholipid antibodies, *Ann. Med.* 29 (1997) 159–163.
- [10] A. Mangia, M. Margaglione, I. Cascavilla, R. Gentile, G. Cappucci, D. Facciorusso, E. Grandone, G. Di Minno, M. Rizzetto, A. Andriulli, Anticardiolipin antibodies in patients with liver disease, *Am. J. Gastroenterol.* 94 (1999) 2983–2987.
- [11] J.F.S. Crocker, K.W. Renton, S.H. Lee, K.R. Rozee, S.C. Digout, D.A. Malatjalian, Biochemical and morphological characteristics of a mouse model of Reye's Syndrome induced by the interaction of influenza B virus and a chemical emulsifier, *Lab. Invest.* 54 (1986) 32–40.
- [12] H.A. Kessler, G.M. Trenholme, A.A. Harris, S. Levin, Acute myopathy associated with influenza A/Texas/1/77 infection. Isolation of virus from a muscle biopsy specimen, *JAMA* 243 (1980) 461–462.
- [13] S. Fujimoto, M. Kobayashi, O. Uemura, M. Iwasa, T. Ando, T. Katoh, C. Nakamura, N. Maki, H. Togari, Y. Wada, PCR on cerebrospinal fluid to show influenza-associated acute encephalopathy or encephalitis, *Lancet* 352 (1998) 873–875.
- [14] E.L. Mazo, V.A. Rusiaev, A.N. Federov, N.G. Iaroslavtseva, I.G. Kharitonov, Effect of the matrix protein of the influenza virus on oxidative phosphorylation in preparations of isolated liver mitochondria from white mice, *Vopr. Virusol.* 33 (1988) 153–157.
- [15] L.E. Davis, Influenza B virus model of Reye's syndrome. Evidence for a nonpermissive infection of liver and brain, *Lab. Invest.* 56 (1987) 32–36.