Prophylaxis with poly(I:C) protects neutropenic mice against *Escherichia coli* meningoencephalitis

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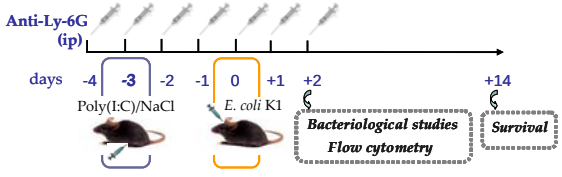
INTRODUCTION and PURPOSE

CNS infections caused by *Escherichia coli*, common in newborns, old and immunocompromised persons, are associated with high rates of mortality and long-term sequelae despite adequate antimicrobial therapy [1]. Polyinosine–polycytidylic acid [poly(I:C)] is a synthetic analogue of viral double-stranded RNA that stimulates Toll-like receptor (TLR)3. [2] TLR3 induces immune responses via the TLR/IL-1 receptor (TIR)-domain containing adaptor protein inducing IFN-β (TRIF) [3].

The protective effect of poly(I:C) administration has been mainly reported against viral infections but few data is available regarding their prophylactic use against bacterial infections. *In vitro*, poly(I:C) increased phagocytosis and intracellular killing of *E. coli* K1 by primary cultures of microglial cells [4]. Here, we assessed the protective properties of poly(I:C) pre-treatment against *E. coli* K1 meningoencephalitis in immunocompromised animals which were depleted of granulocytes.

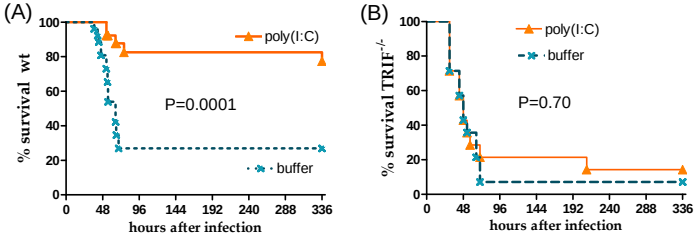
METHODS



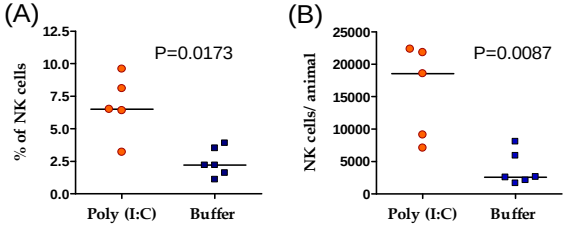
C57BL/6 wild-type (wt) mice were rendered neutropenic by daily intraperitoneal (ip) administration of the anti-Ly-6G mAb (clone 1A8) starting 4 days before infection (with a total of 7 injections). Three days prior to intracerebral infection with *E. coli* K1 ($1x10^4$ CFU/mouse), wt and TRIF$^{-/-}$ (*trif*^{lps2}) mice received an ip injection of either 200 µg poly(I:C) or vehicle (NaCl).

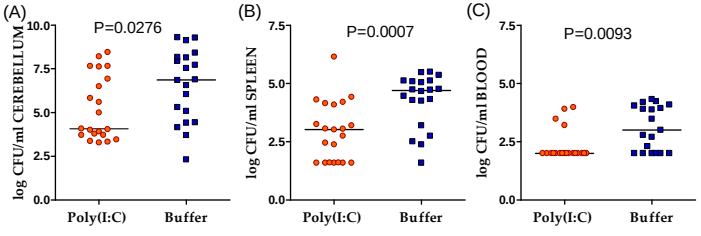
Kaplan-Meier survival curves were analysed by the log-rank test. In bacteriological studies, mice were sacrificed 30 h after infection. Then, bacterial titers and cytokine/chemokine levels of blood/serum, cerebellum and spleen were determined. Also, FACs analysis of the right brain hemisphere containing the site of inoculation was performed. Differences between groups were analysed by Mann-Whitney U-test.

RESULTS



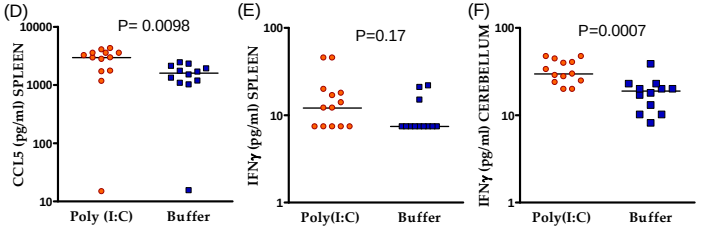
Poly(I:C) protected neutropenic mice from *E. coli* meningoencephalitis in a TRIF-dependent manner.
(A) Pre-treatment with 200 µg poly(I:C) significantly improved survival of neutropenic wt animals. Survival 14 days after infection was 77.4% (16/26) in the poly(I:C)- *vs* 27% (7/26) in the buffer-treated group.
(B) TRIF$^{-/-}$ mice were not protected by prophylaxis with 200 µg poly(I:C). Survival 14 days after infection was 14.3% (2/14) in the poly(I:C)- *vs* 7.1% (1/14) in the buffer-treated group.





Prophylactic administration of poly(I:C) resulted in decreased bacterial loads in (A) cerebellum, (B) spleen and (C) blood, and increased levels of (D) CCL5 and (E,F) IFNγ at early infection. Neutropenic wt mice pre-conditioned with 200 µg poly(I:C) (n=13) or buffer (n=12) were sacrificed 30 h after infection. Each symbol represents an individual mouse. Horizontal bars indicate median values.

The protective effect of poly(I:C) correlated with an increase of (A) the percentage of NK cells ($CD45^+NK1.1^+CD3^-$) and (B) the number of NK cells among $CD45^+$ leukocytes at early infection. Neutropenic wt mice pre-conditioned with 200 µg poly(I:C) (n=5) or treated with buffer (n=6) were sacrificed 30 h after infection. Distinct cell types isolated from the inoculated brain hemisphere from PBS-transcardially perfused animals were analysed by using flow cytometry. Each symbol represents an individual mouse. Horizontal bars indicate median values.



CONCLUSIONS: Prophylactic ip administration of 200 µg of poly(I:C) strengthened the resistance of neutropenic mice against *E. coli* K1 meningoencephalitis. The lack of protection in TRIF-deficient mice strongly suggests that poly(I:C) mediated protection by TRIF signaling pathway.

In correlation with the enhanced survival, poly(I:C) pre-treatment significantly reduced bacterial burdens in cerebellum, spleen and blood at early infection. The protective effect of poly(I:C) was associated with an increased amount and % of NK cells in the brain and higher levels of CCL5 (RANTES) and IFNγ compared to buffer-treated animals.

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