

Supplementary Materials for

Treating murine inflammatory diseases with an anti-erythrocyte antibody

Andrew R. Crow, Rick Kapur, Sandra Koernig, Ian K. Campbell, Chao-Ching Jen, Patrick J. Mott, Danielle Marjoram, Ramsha Khan, Michael Kim, Jennifer Brasseit, Yoelys Cruz-Leal, Alaa Amash, Simrat Kahlon, Issaka Yougbare, Heyu Ni, Adrian W. Zuercher, Fabian Käsermann, John W. Semple, Alan H. Lazarus*

*Corresponding author. Email: lazarusa@smh.ca

Published 21 August 2019, *Sci. Transl. Med.* **11**, eaau8217 (2019)

DOI: [10.1126/scitranslmed.aau8217](https://doi.org/10.1126/scitranslmed.aau8217)

The PDF file includes:

- Fig. S1. Priming with Ter119 can protect mice from Ter119-induced hypothermia.
- Fig. S2. Ter119 substantially reduces the detection of complement components in the joint of arthritic mice.
- Fig. S3. Ter119 and deglycosylated Ter119 induce anemia over time in the CAIA model.
- Fig. S4. Ter119 class-switched to murine IgG1 and murine IgG2a sequences.

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/11/506/eaau8217/DC1)

Data file S1 (Microsoft Excel format). Primary data.

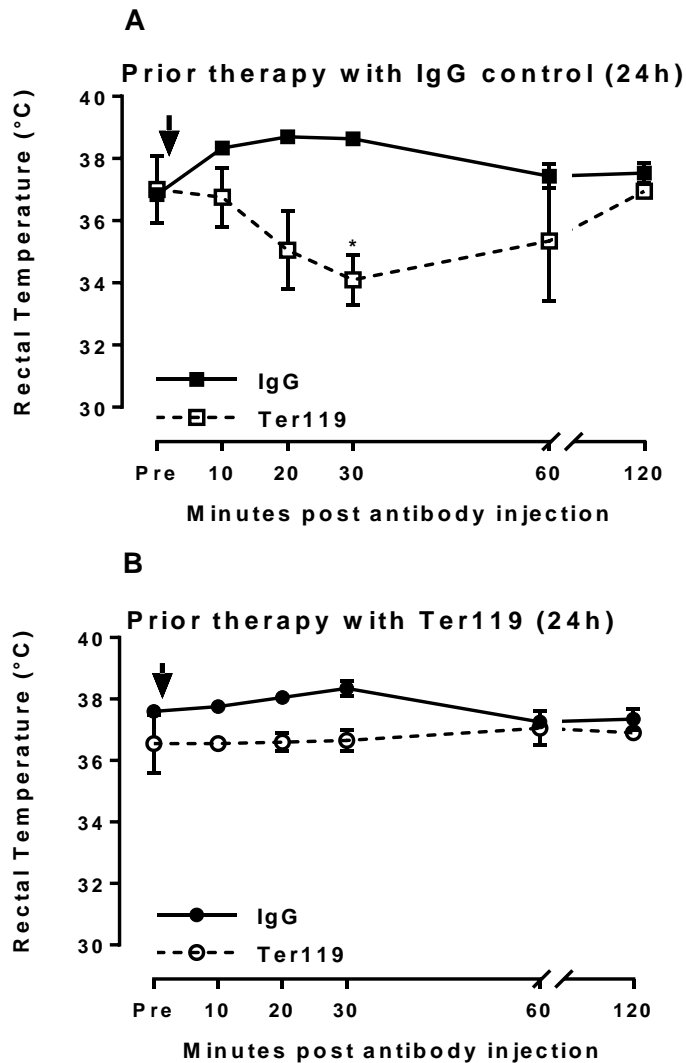


Fig. S1. Priming with Ter119 can protect mice from Ter119-induced hypothermia. The CD-1 mice as used in Fig 1A were returned to their cages for 24 hours. Mice previously injected with the IgG control (A) or previously injected with Ter119 (B) were assessed for rectal temperature (Pre), provided free access to a heating pad for 5 min, and injected with 40 μ g control IgG or Ter119 and rectal temperature taken for 10-120 min. Data are presented as mean \pm SEM; n=2-3 mice from each group (A) or n=2 mice from each group (B). Multiple t test (A & B) with the Holm-Sidak post-test. Significance determined by comparing TER-119 to the rat IgG2b control IgG at each corresponding time point. * P<0.05.

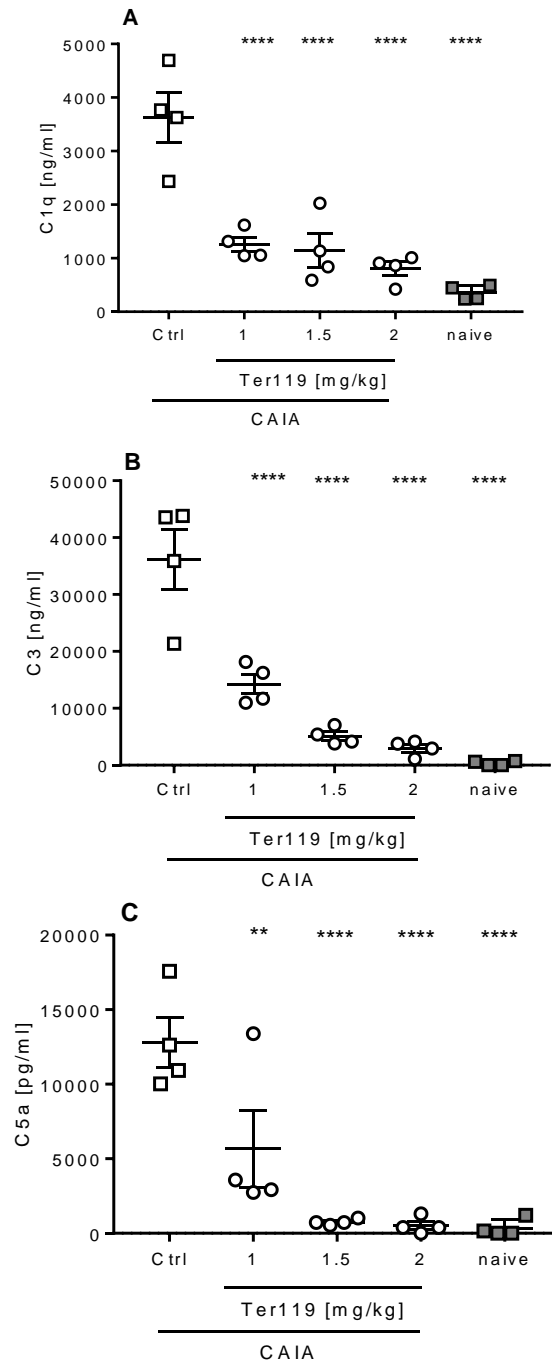


Fig. S2. Ter119 substantially reduces the detection of complement components in the joint of arthritic mice. C57BL/6 mice were injected with the 5 clone monoclonal antibody mixture (i.p.) on day 0 followed by 50 μ g LPS on day 3 to induce CAIA. Arthritis clinical score was evaluated daily (see Fig 4). On day 5 the mice were injected with 2 mg/kg of the isotype control IgG (Ctrl) or Ter119 at the indicated concentrations and on day 6 the complement components C1q (A), C3 (B), and C5a (C) assessed from the joint fluid by ELISA. Data were analysed by a one-way ANOVA test with Holm-Sidak's multiple comparison to the control group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

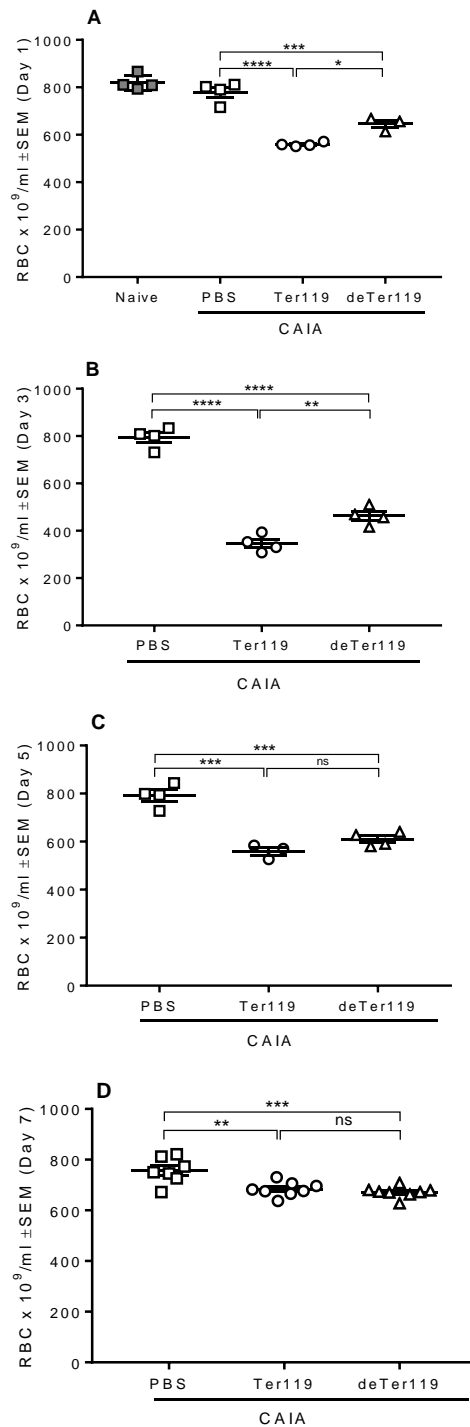


Fig. S3. Ter119 and deglycosylated Ter119 induce anemia over time in the CAIA model. CAIA mice on day 5 were injected with PBS, 1.5 mg/kg of Ter119 or 1.5 mg/kg of deglycosylated Ter119 (deTer119) and erythrocyte concentration evaluated 1 day (A), 3 days (B), 5 days (C), or 7 days (D) after the PBS/Ter119/deglycosylated Ter119 treatments. The data in this supplementary figure is the same as Fig. 6E but with statistics displayed. One-way ANOVA with Tukey's multiple comparison test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

Fig. S4. Ter119 class-switched to murine IgG1 and murine IgG2a sequences. Sequences of heavy and light chains for Ter119 expressed as a mouse IgG1 (A) and mouse IgG2a (B).

A) Ter119 (mouse IgG1, kappa)

Light chain protein sequence:

MGWSCILFLVATATGVHSDIQMTQSPSVLSASVGDRVTLNCKASQNINKYLNWYQQK
LGEAPKVLIIYNTNQLQTGIPSRFSGSGSGTDFTLTISSLQPEDFATYFCFQHYTWPTFGGG
TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNMFYPKDINVKWKIDGSRQNGVLN
SWTDQDSKDYMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNREK

Theoretical pI/Mw: 6.34 / 23680.24

Signal Sequence

VL (Ter119 hybridoma-derived, rat)

CL (mouse Kappa)

Heavy chain protein sequence:

MGWSCILFLVATATGAHSEVKLQESGGGLVQPGGSLKLSCVASGFTFRDHWMNWVR
QAPGKTM EWIGDIRPDGSDTNYAPSVNRNFTISRDNARSILYLQMSNMRSDYTATYYCV
RDSPTRAGLMDAWGQGTSTVSSAKTTAPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE
PVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTPSSTWPSETVTCNVAHPASSTKVD
KKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLITLTPKVTCVVVDISKDDPEVQFSWF
VDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISK
TKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPI
MDTDGSYFVYSKLVNPKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK

Theoretical pI/Mw: 6.82 / 50976.73

Signal Sequence

VH (Ter119 hybridoma-derived, rat)

CH (mouse IgG1)

B) Ter119 (mIgG2a, kappa)

Light Chain protein sequence:

MGWSCII~~FLVATATGVHS~~DIQMTQSPSVLSASVGDRTLNCKASQNKYLNWYQQKLGEPK
VLIYNTNNLQTGIPSRFSGSGGTDFTLTISSLQPEDFATYFCFQHYTWPTFGGGTKLEIKRADAAP
TVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSST
LTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

Theoretical pI/Mw: 6.34 / 23680.24

Signal Sequence

VL (Ter119 hybridoma derived, rat)

CL (mouse Kappa)

Heavy Chain protein sequence:

MGWSCII~~FLVATATGAHSE~~VKLQESGGGLVQPGGSLKLSCLVSGFTFRDHWMNWRQAPGKT
MEWIGDIRPDGSDTNYAPSVNRFTISRDNARSILYLQMSNMRSYDTATYYCVRDSPTTRAGLMD
AWGQGTSVTVSSAKTTAPSVYPLAPVCGDITGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHT
FPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKIEPRGPTIKPCPPCKCPAPNLLG
GPSVFIFPPKIKDVLMI~~SLPIVTCVVVDVSEDDPDVQISW~~FVNNVEVHTAQTQTHREDYNSTLRV
VSALPIQHQQDWM~~SGKEFKCKV~~NNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTC
MVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDS~~DGSYFMYSKLR~~VEKKNWVERNSYSCSVVH
EGLHNHHTTKSFSRTPGK

Theoretical pI/Mw: 7.98 / 49691.34

Signal Sequence

VH (Ter119 hybridoma derived, rat)

CH (mouse IgG2a)