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Complement-producing maternal microchimeric cells override infection susceptibility in complement-deficient murine offspring

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To the editor: Long-term persistence of vertically transferred maternal cells occurs ubiquitously in mammalian offspring (1). Presence of these exceptionally rare maternal microchimeric cells (MMc), with ensuing immunological tolerance to noninherited maternal antigen (NIMA), is associated with a variety of remarkable phenotypes including serological resistance to noninherited maternal HLA sensitization (2), improved long-term survival of NIMA-matched renal allografts (3), neonatal heart block (4), type 1 diabetes (5), and cross generational reproductive fitness with expanded accumulation of NIMA-specific regulatory T cells (6). Herein, we considered whether MMc may exert other physiological benefits beyond these immunological features linked with antigenicity.

A provocative consideration is whether phenotypically wildtype MMc can reduce disease severity in autosomal recessive disorders caused by defective or missing proteins. Given shared susceptibility to infection caused by complement deficiency in humans and mice (7), and enriched MMc in the liver where C3 and other complement components are produced (8), this hypothesis was investigated by evaluating complement levels and infection susceptibility of C3

NIMA (C3-/- mice born to C3+/- mothers) compared with genetically identical C3-/- mice born to complement deficient mothers, along with C3+/- littermate controls (**Figure 1A**; **Supplemental Figure 1**).

We found increased serum C3 levels in C3 NIMA compared with C3-/- mice born to complement deficient mothers, albeit at levels still considerably reduced compared with C3+/- controls (**Figure 1B**). C3-/- mice are highly susceptible to *E. coli*, and an intermediate dosage (15000 CFUs) that accentuates this susceptibility was used for intravenous infection to further investigate functional consequences of complement producing MMc (**Supplemental Figure 2**). These experiments showed the normally high bacterial burden in tissues of infected C3-/- mice were reduced in C3 NIMA mice (**Figure 1C**), demonstrating that being born to complement sufficient mothers can dominantly impact infection susceptibility of otherwise genetically identical complement-deficient mice.

To verify importance of C3+/- MMc, we evaluated C3 levels and infection susceptibility after MMc depletion using antibody or pregnancy induced MMc displacement in male and female C3 NIMA mice, respectively. For antibody MMc depletion, transgenic mice with constitutive cell surface expression of ovalbumin (OVA) (6, 9) were intercrossed with C3-/- mice to generate C3 OVA NIMA offspring born to C3+/-OVA+/- mothers (**Supplemental Figure 3**). Transforming OVA with C3 into NIMAs in this fashion allows MMc depletion using anti-OVA IgG, and verifying loss of MMc by quantifying OVA+ genomic DNA in tissues such as heart, liver and uterus which consistently contain highest MMc levels (6, 9). These experiments showed C3+/-OVA+/- MMc depletion reduces serum C3 to background levels (**Figure 1B**), and overturns infection resistance of C3 NIMA mice (**Figure 1C, 1D**).

Despite the ability to persist long-term, MMc are also susceptible to pregnancy induced displacement and replacement with fetal microchimeric cells (FMc) (9). To further investigate the necessity of C3+/- MMc in C3 NIMA females, we compared C3 levels and susceptibility after pregnancy sired by complement-deficient males with ensuing replacement by C3-/- FMc. C3 NIMA female mice postpartum after pregnancy sired by C3-/- males, with loss of C3+/- MMc, contained only background serum C3 levels (**Figure 1B**), and infection susceptibility comparable to C3-/- controls (**Figure 1E, 1F**). Thus, complement producing MMc are

responsible for above background C3 levels and reduced infection susceptibility in complement deficient offspring.

Beyond complement deficiency, these results suggesting clinical phenotypes associated with missing or defective proteins in autosomal recessive disorders can be altered by functionally wildtype MMc opens up fundamental new ways for explaining why individuals with the same gene defect in many autosomal recessive disorders, including cystic fibrosis and sickle cell anemia, have widely varied disease severity (10, 11). In turn, these protective benefits associated with complement producing MMc highlight importance for further investigating how these cells work, including their cellular identity and phenotype heterogeneity, since expanding their accumulation beyond natural microchimeric levels represents an innovative approach for therapeutically reducing severity of common genetic disorders.

REFERENCES

1. Nelson JL. The otherness of self: microchimerism in health and disease. *Trends Immunol.* 2012;33(8):421-7.
2. Claas FH, Gijbels Y, van der Velden-de Munck J, and van Rood JJ. Induction of B cell unresponsiveness to noninherited maternal HLA antigens during fetal life. *Science.* 1988;241(4874):1815-7.
3. Burlingham WJ, Grailer AP, Heisey DM, Claas FH, Norman D, Mohanakumar T, et al. The effect of tolerance to noninherited maternal HLA antigens on the survival of renal transplants from sibling donors. *N Engl J Med.* 1998;339(23):1657-64.
4. Stevens AM, Hermes HM, Rutledge JC, Buyon JP, and Nelson JL. Myocardial-tissue-specific phenotype of maternal microchimerism in neonatal lupus congenital heart block. *Lancet.* 2003;362(9396):1617-23.
5. Nelson JL, Gillespie KM, Lambert NC, Stevens AM, Loubiere LS, Rutledge JC, et al. Maternal microchimerism in peripheral blood in type 1 diabetes and pancreatic islet beta cell microchimerism. *Proc Natl Acad Sci U S A.* 2007;104(5):1637-42.
6. Kinder JM, Jiang TT, Ertelt JM, Xin L, Strong BS, Shaaban AF, et al. Cross-Generational Reproductive Fitness Enforced by Microchimeric Maternal Cells. *Cell.* 2015;162(3):505-15.
7. Wessels MR, Butko P, Ma M, Warren HB, Lage AL, and Carroll MC. Studies of group B streptococcal infection in mice deficient in complement component C3 or C4 demonstrate an essential role for complement in both innate and acquired immunity. *Proc Natl Acad Sci U S A.* 1995;92(25):11490-4.
8. Alper CA, Johnson AM, Birtch AG, and Moore FD. Human C'3: evidence for the liver as the primary site of synthesis. *Science.* 1969;163(3864):286-8.

9. Shao TY, Kinder JM, Harper G, Pham G, Peng Y, Liu J, et al. Reproductive outcomes after pregnancy-induced displacement of preexisting microchimeric cells. *Science.* 2023;381(6664):1324-30.
10. Tewari S, Brousse V, Piel FB, Menzel S, and Rees DC. Environmental determinants of severity in sickle cell disease. *Haematologica.* 2015;100(9):1108-16.
11. Drumm ML, Ziady AG, and Davis PB. Genetic variation and clinical heterogeneity in cystic fibrosis. *Annu Rev Pathol.* 2012;7:267-82.

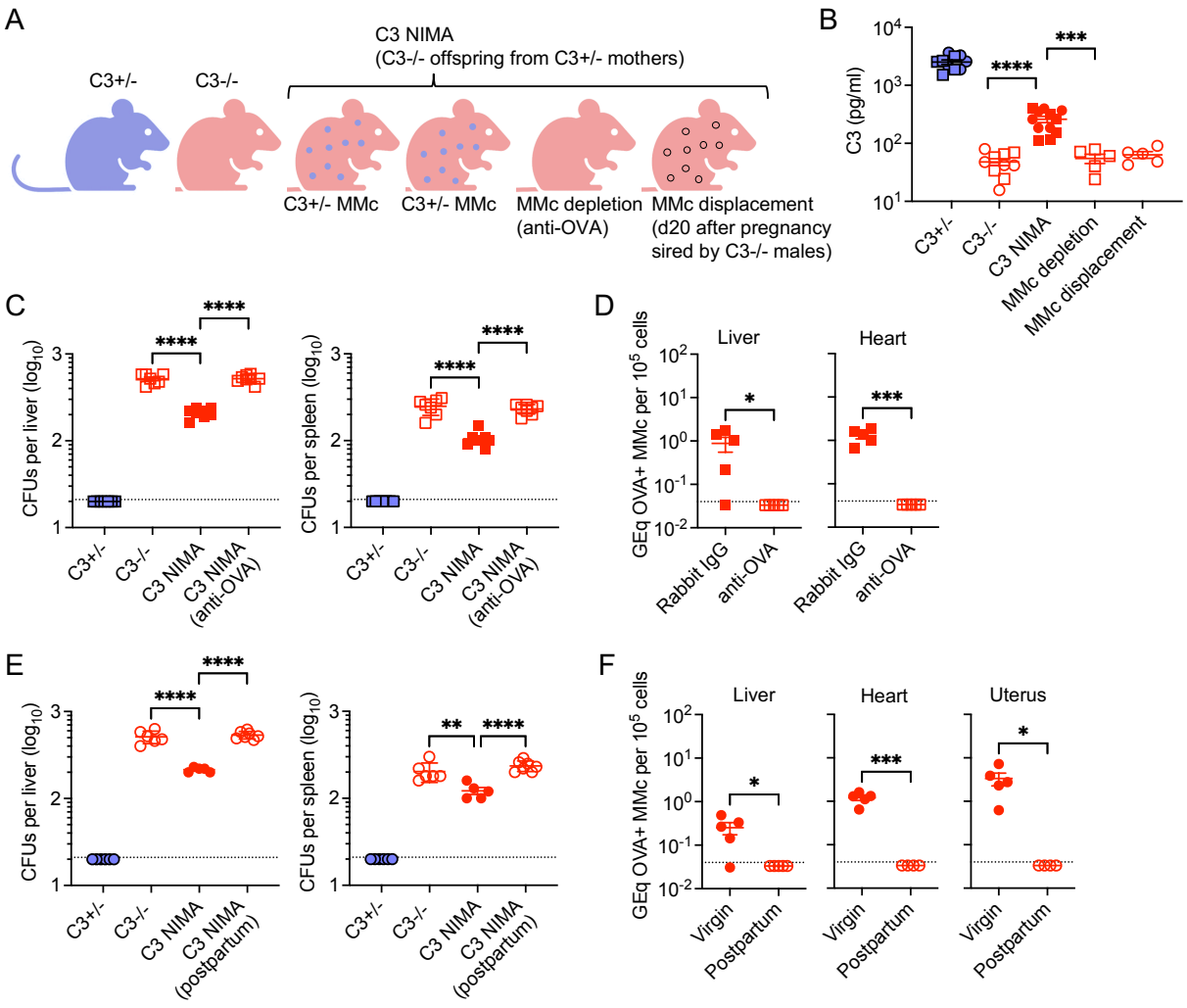

Figure 1. C3+/- MMc overrides complement deficiency in C3-/- mice. (**A**) Schematic comparing C3+/-, C3-/- and C3 NIMA mice, or C3 NIMA mice after MMc depletion or displacement. (**B**) Serum C3 levels in male (square) or female (circle) mice described in panel A. (**C**) *E. coli* CFUs after infection for male C3+/-, C3-/-, C3 NIMA and C3 NIMA mice depleted of OVA+ MMc using anti-OVA IgG. (**D**) Genome equivalents (GEq) OVA DNA specific to OVA+ MMc in male C3 OVA NIMA mice 14 days after anti-OVA compared with isotype control IgG administration. (**E**) *E. coli* CFUs after infection for female C3+/-, C3-/-, virgin C3 NIMA and C3 NIMA postpartum after pregnancy sired by C3-/- males. (**F**) GEq OVA DNA specific to OVA+ MMc among female C3 OVA NIMA mice 20 days postpartum after pregnancy sired by C3-/- males compared with age matched virgin control mice. Each point represents the data from an individual mouse, combined from at least 2 independent experiments each with similar results. Bar, mean ± standard error. **P*<0.05; ***P*<0.01; ****P*<0.005; *****P*<0.001.