Hobit. We identified 4510 genome-wide binding sites for Blimp1, which could be assigned to 3241 genes. Hobit bound 404 sites, >50% of which overlapped with Blimp1 (fig. S17). Note that 78% of the 159 genes bound by Hobit were also bound by Blimp1, whereas 31% of the genes deregulated in the absence of Hobit and Blimp1 were bound by Hobit, Blimp1, or both (Fig. 4E). Hobit and Blimp1 used nearly identical binding motifs (Fig. 4E), which supports the notion of extensive cooperation between both factors. Hobit and Blimp1 bound target sequences within the KLF2 locus (Fig. 4G). Thus, Hobit and Blimp1 mediate a transcriptional program of tissue residency that is shared among Trm, trNK, and NKT cells and includes the suppression of tissue egress genes.

The recent recognition of Trm cells as essential sentinels at barrier tissues (27–30) highlights the importance of understanding their development. We have characterized a universal transcriptional program of tissue residency that is shared between adaptive and innate lymphocytes at both epithelial and nonepithelial sites. Our findings reveal the central roles of Hobit and Blimp1 in the establishment of tissue residency and indicate that they function by silencing lymphocyte egress from the tissues and by repressing the development of circulating memory cells. The key insights into the transcriptional regulation of Trm cells within this study may assist in the manipulation of tissue-resident memory for the development of vaccine strategies to improve localized immunity.

REFERENCES AND NOTES


Supplementary materials. RNA and ChIP sequencing data have been made available under accession numbers GSE70813 and GSE79339 at GEO. Blimp1-Bio mice are available from M. B. under a material transfer agreement (MTA) with IMBL, Blimp1-GFP and Blimp1+Hobit DKO mice are available from A.K. under an MTA with The Walter and Eliza Hall Institute and Hobit KO and Blimp1+Hobit DKO mice are available from K.P.J.M.v.G. under an MTA with Sanquin Research. L.K.M. was supported by grant DEL41001432 from the ARC and grant 1083685 from the National Health and Medical Research Council of Australia (NHMC). M.M. and M.B. were supported by the Boehringer Ingelheim and a European Research Council Advanced Grant (291740-LymphoControl) from the European Community’s Seventh Framework Programme. A.B. was funded by a Fellowship from the German Research Foundation. R.S. was supported by a Fellowship from the Alexander von Humboldt Foundation. W.S. was supported by grant 1023454 from the NHMRC. N.A.M.K. and R.A.W.v.L. were supported by grant 1136 of the Landsteiner Foundation of Blood Transfusion Research. D.I.G. was supported by an NHMRC Early Career Fellowship (1054431), and D.I.G. was supported by an NHMRC Senior Principal Research Fellowship (1020770). G.T.B. was supported by a fellowship from the ARC and grant 1042582 from the NHMRC. A.K. was supported by a fellowship from the Sylvia and Charles Viertel Foundation and a project grant (673345) from the NHMRC. F.M.B. and K.P.J.M.v.G. were supported by Vidi grant 917.13.338 from The Netherlands Organization of Scientific Research. This work was supported by the Victorian State Government Operational Infrastructure Support and Australian Government NHMRC Independent Research Institute Infrastructure Support scheme.

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INNATE IMMUNITY

Mx1 reveals innate pathways to antiviral resistance and lethal influenza disease

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Influenza A virus (IAV) causes to half a million deaths worldwide annually, 90% of which occur in older adults. We show that IAV-infected monocytes from older humans have impaired antiviral interferon production but retain intact inflammasome responses. To understand the in vivo consequence, we used mice expressing a functional Mx gene encoding a major interferon-induced effector against IAV in humans. In Mx2-intact mice with weakened resistance due to deficiencies in Mavs and Tlr7, we found an elevated respiratory bacterial burden. Notably, mortality in the absence of Mavs and Tlr7 was independent of viral load or MyD88-dependent signaling but dependent on bacterial burden, caspase-1/11, and neutrophil-dependent tissue damage. Therefore, in the context of weakened antiviral resistance, vulnerability to IAV is a function of caspase-dependent pathology.

Respiratory infections with influenza A virus (IAV) cause severe morbidity and mortality in humans and animals worldwide. Older humans are highly susceptible to influenza disease. This susceptibility could be due to an inability to mount an effective antiviral response or an incapacity to develop disease tolerance to IAV infection (1–4). We began by comparing the innate immune pathways engaged by IAV infections in peripheral blood monocytes from young-adult (20- to 30-year-old) and older (65- to 89-year-old) human donors (table S1 and fig. S1A). IAV-infected monocytes from older humans showed intact nuclear factor κB (NF-κB)-dependent proinflammatory cytokine expression and secretion [interleukin (IL)–6] (fig. S1B and Fig. 1A) and robust inflammasome-dependent cytokine expression and secretion (IL-1β) (fig. SIC and Fig. 1B). Type I interferon (IFN) responses to IAV infection, however, were significantly attenuated in older human monocytes (IFN-β) (Fig. 1C). Reduced

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IFN secretion from older monocytes was not attributable to the expression level of RIG-I, a viral RNA sensor that induces the type I IFN responses that drive antiviral immunity (5, 6) (fig. S1D) or to a particular demographic group (fig. S1, E to G). Similar age-dependent reduction in IFN-β was observed from monocytes transfected with ligands of RIG-I (fig. S2, A to F) and from macrophages stimulated with polyinosinic-polyribydlic acid (fig. S2, G to I). These data indicate that older human monocytes and macrophages have intact RIG-I signaling to activate proinflammatory cytokines and the inflammasome but have impaired signaling to induce type I IFNs.

Comparison of a broader array of genes revealed that infected monocytes from older donors showed consistently lower expression of several IFN-stimulated genes (ISGs) (MxA, IFITM2, and ISG15, all of which are known to have antiviral activities for influenza viruses) (fig. 1D). Furthermore, expression of IRF7, a critical transcription factor for type I IFNs, and STAT1, a type I IFN receptor signaling molecule, was lower in the older cohort. Consequently, older monocytes infected with IAV expressed higher levels of influenza viral genes (NS and M) (fig. 1D). Selective impairment in IFN responses to other viral infections (7), after vaccination against influenza (8) or downstream of TLR7 (4, 9, 10), suggest that decreased IFN and elevated proinflammatory cytokines are a common characteristic of the aging innate immune system in humans.

To probe the possible in vivo consequences of weak IFN responses in the face of robust inflammation after IAV infection, we sought to employ a relevant mouse model. Older C57BL/6 mice did not show increased susceptibility to IAV infection over young mice; in fact, older mice showed antiviral resistance (fig. S3), failing to phenocopy aging human infections (11). We therefore decided to mimic human innate effector responses using genetic approaches to determine the consequences of the loss of pattern recognition receptors (PRRs) that induce IFNs during IAV infection. Inbred mouse strains, including C57BL/6, carry non-functional alleles of the Mx1 gene. The myxovirus resistance protein 1 (Mx1) is a dynamin-like guanosine triphosphatase that blocks primary transcription of influenza, presumably by binding to viral nucleoproteins (12–14). We predicted that intact Mx1 would affect the dynamics of viral spread and the host response to IAV infection, and that Mx1-sufficient mice would more closely model influenza pathogenesis in humans. As reported previously (15, 16), Mx1 congenic mice on the C57BL/6 background were highly resistant to IAV (A/PR8) infections as compared with...
Mx1-deficient C57BL/6 mice (Fig. S4). The observed resistance in Mx1 congenic mice was not due to more robust adaptive immunity because these mice showed low levels of T and B cell-mediated responses to IAV, likely because of rapid viral clearance (Fig. S4, D and E).

We therefore used Mx1 mice to investigate the relative contributions of the innate immune-sensing pathways during IAV infection: Toll-like receptors (TLRs) and RIG-I, which both induce type I IFNs (5), and the inflammasome pathway that activates caspase-1, releases IL-1β and IL-18, and induces pyroptosis (17, 18). At a high dose of viral challenge [10^6 plaque-forming units (PFU)], Tr7−/− or Casp1/11−/− mice survived infection, whereas Mavs−/− and Tr7−/− × Mavs−/− mice lost weight and died by day 7 or day 5, respectively, with a high viral burden (Fig. S5). After challenge with a lower dose of A/PR8 virus (100 PFU), Tr7−/−, Mavs−/−, and Casp1/11−/− mice were resistant, but Tr7−/− × Mavs−/− double-deficient mice lost weight and succumbed to infection (Fig. 2, A and B). The mechanism of protection conferred by MAVS and TLR7 in the Mx1-sufficient host correlated with the ability of these PRRs to induce IFN-β secretion (Fig. 2C). In contrast, inflammatory cytokine secretion was observed independently of MAVS and TLR7 (fig. S6). Mice deficient in both Mavs and Tlr7 lost Mx1 expression (Fig. 2D) and therefore succumbed.
to IAV challenge. Thus, unlike C57BL/6 mice (J9–22), MX1 congenic mice reveal the key innate sensors that confer antiviral resistance, RIG-I and TLR7; these sensors are responsible for the production of type I IFNs that induce Mx1 expression and potentely control IAV replication. This is consistent with our results in monocytes from older humans, where impaired RIG-I signaling led to low IFN induction and compromised innate IAV resistance (Fig. 1).

To assess the role of inflammasome activation in the context of an impaired interferon response, we generated congenic Tlr7−/− × Mx1−/− Casp1/11−/− mice. Strikingly, these mice exhibited protection from IAV disease (Fig. 3A and B) despite having viral loads similar to Tlr7−/− × Mavs−/− mice (Fig. 3C and fig. S8A). Improved protection was therefore not a function of greater antiviral resistance but a lack of Casp1/11 activity. Tlr7−/− × Mavs−/− × Casp1/11−/− mice eventually cleared the virus by 30 days after infection (fig. S8B). Tlr7−/− × Mavs−/− mice also succumbed to infection with a human isolate of the 2009 pandemic strain of H1N1 (pH1N1) faster than other genotypes at two days postinfection, and a separate function of caspase-1/11, led to low IFN induction and compromised innate immunity in the lung after intranasal inoculation of bacterial lipopolysaccharide (LPS) (fig. S13), indicating that the requirement of Casp1/11 for NET release extends beyond IAV infection. NET digestion by treatment with deoxyribonuclease (DNase) (Pulmozyme) resulted in prolonged survival of Tlr7−/− × Mavs−/− mice after IAV infection (Fig. 4A). Thus, in the absence of Tlr7 and Mavs, NETosis contributes to mortality after IAV infection, likely in part by inducing collateral tissue damage (28).) Despite the lack of innate viral control, depletion of neutrophils or degradation of NETs can induce disease tolerance to influenza.

Older adults (>65 years of age) are most vulnerable to the flu, and many succumb to pneumonia caused by secondary bacterial infections (29, 30). IAV infection and consequent airway epithelial damage are sufficient to enhance bacterial colonization of the lungs (31–35). Therefore, we hypothesized that IAV-induced disease and mortality in the Tlr7−/− × Mavs−/− mice may be secondary to bacterial infection. Notably, bacterial bloom was observed in the nasal cavity of infected Tlr7−/− × Mavs−/− and Tlr7−/− × Mavs−/− × Casp1/11−/− mice, as compared with the IAV-resistant wild-type mice (Fig. 4, C and D). In addition, an increase in the abundance of Pasteurella were observed in the nasal cavities (Fig. 4E) and lungs (Fig. 4F and G) of IAV-infected Tlr7−/− × Mavs−/− and Tlr7−/− × Mavs−/− × Casp1/11−/− mice. Finally, combination antibiotic treatment beginning 3 days after IAV infection partially rescued Tlr7−/− × Mavs−/− mice from lethality (Fig. 4H). Collectively, these data indicate that a failure to induce type I IFNs promotes viral amplification and tissue damage within the respiratory environment, conducive to bacterial bloom. Neutrophil recruitment and caspase-dependent NETosis contributes to lethality. These results in Mx1 congenic mice are consistent with the notion that age-related defects in innate immunity (reduced IFN responses) could predispose IAV-infected older adults to secondary bacterial infection. A direct implication of our findings is that older adults suffering from IAV infection may benefit from therapeutic strategies that minimize inflammatory responses mediated by neutrophils.

REFERENCES AND NOTES

SUPPORTING MATERIALS
www.sciencemag.org/content/352/6284/463/suppl/DC1
Materials and Methods
Table S1
Figs. S1 to S11
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Flu immunity shows its age

As we age, our immune systems change; in many ways not for the better. For instance, the elderly account for 90% of influenza deaths annually. Pillai et al. now report that influenza-infected human monocytes, a type of immune cell, exhibit reduced antiviral activity. In influenza-infected mice, two innate immune sensing pathways work together to promote antiviral immunity to influenza. Mice lacking antiviral immunity (similar to the situation in elderly people) had elevated bacterial burdens in their lungs and increased inflammatory responses, which both contributed to their increased susceptibility to influenza.

Science, this issue p. 463