Interferon regulatory factor (IRF) regulation of the type I interferon response has not been extensively explored in murine retroviral infections. IRF-3−/− and select IRF-3/7−/− mice were resistant to LP-BM5-induced pathogenesis. However, further analyses strongly suggested that resistance could be attributed to strain 129-specific contamination of the known retrovirus resistance gene Fv1. Therefore, caution should be taken when interpreting phenotypes observed in these knockout mice, as strain 129-derived genetic polymorphisms may explain observed differences.

The type I interferon (IFN) antiviral response (IFN-α and IFN-β) plays a major role in host innate immunity to RNA and DNA virus infections (1–12). This antiviral type I IFN response is tightly regulated, in part, by interferon regulatory factors (IRFs) via upstream signaling induction by various pattern recognition receptors (PRRs). Several studies utilizing knockout mice for IRF-3, IRF-7, and IRF-3/7 (DKO) have reported on the overall complexity and sometimes distinct roles of IRF-3 and IRF-7 in antiviral IFN-dependent and IFN-independent responses to multiple viral infections (1–3, 9, 10, 13–16). Although most retroviruses are relatively poor inducers of robust innate antiviral responses, there is growing evidence for the existence of retrovirus-induced type I IFNs; therefore, IRF-3 and/or IRF-7 may also be involved (17–23).

LP-BM5, a gammaretrovirus, causes murine AIDS (MAIDS) upon infection of susceptible mouse strains—e.g., wild-type C57BL/6 (B6) (24, 25). MAIDS is characterized by early activation parameters, followed by broad, profound immunodeficiency of both T- and B-cell responses and susceptibility to opportunistic microbial infection (24–34).

To determine the possible role of IRFs in retroviral pathogenesis, B6 background mouse strains with IRF-3−/−, IRF-7−/−, and DKO congenic status (10, 13, 35) were infected with 5 × 10⁴ PFU of LP-BM5 retrovirus and were compared to MAIDS-susceptible B6 and MAIDS-resistant 129S1/SvImJ (129) mice (NCI, Bethesda, MD) (31, 36). Upon sacrifice, at 8 weeks postinfection (wpi), MAIDS was assessed (Fig. 1). The extent of pathogenesis was calculated from established disease parameters, and a disease index was calculated: gray symbols, uninfected mice; open or black symbols, mice assayed 8 wpi. Solid gray horizontal lines indicate means. *, P < 0.05; **, P < 0.01 (Mann-Whitney test).

FIG 1 IRF-3−/− mice demonstrate increased MAIDS resistance. IRF-3−/−, IRF-7−/−, DKO, B6, and 129 mice were infected with LP-BM5, and MAIDS disease index was calculated: gray symbols, uninfected mice; open or black symbols, mice assayed 8 wpi. Solid gray horizontal lines indicate means. *, P < 0.05; **, P < 0.01 (Mann-Whitney test).
was assigned, with disease severity ranging from 0 (no disease) to 5 (most-severe disease) (37–39). Infected IRF-3/IRF-7 mice were almost as resistant as MAIDS-resistant 129 mice, whereas IRF-7 mice developed disease equivalent to that seen with the susceptible B6 controls. Surprisingly, DKO mice succumbed to variable and intermediate disease, with some resistant mice, which was an unexpected result versus previous studies reporting increased susceptibility to infection with other viruses in IRF-3/IRF-7 and/or IRF-

FIG 2 Strain 129 genetic contamination in IRF-3/IRF-7 and DKO mice. IRF-3/IRF-7 and DKO breeder mice were assessed at the DartMouse Speed Congenic Core Facility for overall genetic background in comparison to B6 and 129 mice (A) and SNP genetic analysis of individual chromosomes (Chr) (B). Additional strain 129 contamination on the distal portion of chromosome 4, the site of the Fv1 gene (enlarged box), of IRF-3/IRF-7 and DKO mice is indicated.

FIG 3 Strain 129-specific contamination of Fv1 of IRF-3/IRF-7 and DKO mice. Fv1 genotypes were determined with PCR and visualized by gel electrophoresis using genomic DNA from male (M) and female (F) B6, 129, IRF-3/IRF-7, and DKO breeder mice. Expected band lengths: Fv1b, 621 bp; Fv1nr allele, 406 bp.
7−/− mice and equal or greater susceptibility in DKO mice (3, 10, 13, 14).

Therefore, the genetic backgrounds of breeder knockout mice were assessed for strain 129 genetic material originating from the original knockout construction (27, 40, 41). Upon high-density single nucleotide polymorphism (SNP) analysis (42), IRF-7−/− mice exhibited ~2% strain 129 contamination, consistent with full, standard backcrossing (Fig. 2A). However, IRF-3−/− (15% to 25%) and DKO (10% to 20%) mice had substantial 129 contamination (Fig. 2A); the intermediate contamination level of the DKO mice expected from their derivation by crossing IRF-3−/− and IRF-7−/− mice (10).

The Fv1 gene restricts murine leukemia viruses (MLVs), including LP-BM5 (27, 40, 43, 44), with the Fv1b, Fv1n, and Fv1nr alleles restricting N-tropic, B-tropic, and B- and N-tropic MLVs, respectively (27, 41, 45, 46). To ensure successful B-tropic LP-BM5 infectivity, our studies were performed with homozygous Fv1b/b (B6) mice. SNP analysis of IRF-3−/− and DKO mice identified 129 contamination, in part, at chromosome 4 (distal) in the area of Fv1 (Fig. 2B) (47, 48). Engineered PCR primers specific for Fv1 alleles allowed definition of IRF-7−/− breeders as uniformly Fv1b/b, in contrast, IRF-3−/− breeders were defined as Fv1nr/nr, marking these strains as permissive versus nonpermissive, respectively, to LP-BM5 infection (Fig. 3). DKO breeders were mixed, with Fv1b/b (susceptible) versus Fv1nr/nr and Fv1nr/nr (resistant) DKO progeny (Fig. 3).

DKO progeny mice from controlled matings were produced to allow segregation of the Fv1b and Fv1nr alleles, genotyped, and infected with LP-BM5 to potentially correlate susceptibility and resistance phenotypes with the Fv1 genotype rather than with knockout of IRF-3 (Fig. 4). The disease index determined at 8 wpi, in comparison to susceptible B6 mice, demonstrated that (i) DKO Fv1b/b mice displayed equivalent disease and equal viral load (ectropic gag and defective gag) (data not shown); (ii) infected DKO Fv1nr/nr and DKO Fv1b/b mice, each with at least one resistant Fv1nr allele, displayed significantly less disease; and (iii) there was a single outlier mouse in each ("X" with less disease and "Y" with more disease) for which SNP analysis revealed (~15%) 129 contamination within the range of DKO mice as a whole (data not shown) (Fig. 4). These DKO data thus strongly associate MAIDS pathogenesis with the observed strain 129 contamination and Fv1 allele(s) in a manner independent of the status of the IRF-3 gene.

Thus, on the basis of comparisons to LP-BM5-infected IRF-7−/− and B6 mice, we describe increased MAIDS resistance in infected IRF-3−/− and certain DKO mice, apparently a result of strain 129-derived contamination of the Fv1dr allele in purportedly fully backcrossed (to B6) congenic IRF-3−/− mice. The inconsistent degrees of B6 genetic background in IRF-3−/−, IRF-7−/−, and DKO mice provide a cautionary note that results seen with these mice, in our studies and in those of others, may not be indicative solely of the knockout of the IRF gene(s) itself. The DKO mice have been bred at least three times independently from the single knockout mice, according to previous reports (3, 10, 13, 15, 35), and were previously reported to be of 91% B6 background, versus 97% for the IRF-7−/− mice, via analysis of microsatellite markers (15). These levels of contamination are similar to the B6 background levels observed here by SNP analyses: >98% in IRF-7−/−, 75% to 85% in IRF-3−/−, and 80% to 90% in DKO mice. The incomplete backcrossing to B6 of the IRF-3−/− mice and the 129-derived restrictive Fv-1dr inheritance notwithstanding, the results from the two outlier DKO mice (Fig. 4) suggest the presence of additional strain 129-derived genetic elements that may also affect LP-BM5 infectivity or pathogenesis.

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