HHS Public Access

Author manuscript

Nat Immunol. Author manuscript; available in PMC 2015 July 21.

Published in final edited form as:

Nat Immunol. ; 13(3): 197–198. doi:10.1038/ni.2232.

Oral ivermectin as an unexpected initiator of CreT2-mediated deletion in T cells

Evann Corbo-Rodgers[1], **Elizabeth S. Staub**[1], **Tao Zou**[2], **Abigail Smith**[3], **Taku Kambayashi**[2], and **Jonathan S. Maltzman**[1]

[1]Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

[2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

[3]Department of Pathobiology, School of Veterinary Medicine at the University of Pennsylvania, Philadelphia, PA 19104

To the editor

Ivermectin is a broad-spectrum antihelmintic drug used to treat pinworms and fur mites in laboratory mouse colonies. Here we report activation of a tamoxifen-regulated Cre fusion protein as an unintended but immunologically relevant side effect of oral ivermectin treatment.

Ectoparasites and their treatment represent a growing dilemma in laboratory mouse colonies[1,2]. Topical or oral ivermectin is an effective treatment of fur mites and pinworms because it selectively binds to the glutamate-gated chloride ion channels in muscle and nerve cells, leading to hyperpolarization of the cells, paralysis and parasite death[3]. Ivermectin is generally considered to be safe for rodent colonies, but may alter immune responses[4] and has neurotoxicity in susceptible strains[5].

During a facility wide treatment for mite infestation, we noted unexpected deletion of the *lox*P-STOP-*lox*P cassette of the *Rosa26*^{YFP} Cre reporter (R26R^{YFP})[6] in two independent colonies of mice that transgenically expressed a tamoxifen-regulated Cre (UBC-CreT2)[7]. Normally the CreT2 fusion protein, transcriptionally regulated here by the ubiquitin promoter, is inactive unless bound specifically to 4-hydroxytamoxifen (4-OHT) or ICI 182,780 (ICI)[8]. In greater than five years of experience with the UBC-CreT2 and R26R^{YFP} strains, we have not previously noted CreT2-mediated deletion in hematopoietic lineages without tamoxifen exposure (data not shown and [9]).

Our breeding strategy segregates floxed alleles from Cre transgenics in parents; specifically, R26R$^{YFP/YFP}$ were intercrossed with UBC-CreT2$^{+/-}$ to generate R26R$^{YFP/+}$UBC-CreT2^{+} offspring. Following initiation of ivermectin-containing feed (12 ppm), we were surprised to

Correspondence should be addressed to J.S.M (maltz@mail.med.upenn.edu), Jonathan S. Maltzman, 754 BRB II/III, 421 Curie Blvd., Philadelphia, PA 19104, Phone: 215-573-1833, Fax: 215-573-7599.

find that approximately 60% of $R26R^{YFP}$UBC-$CreT2^{+}$ offspring expressed yellow fluorescent protein (YFP) in peripheral blood cells (Fig. 1a). Deletion was not confined to the *Rosa26* locus; multiple additional floxed loci present in our colony underwent recombination in UBC-$CreT2^{+}$ ivermectin-treated mice (data not shown). Breeding cages continued to generate offspring with YFP^{+} cells more than fifteen weeks following cessation of ivermectin. Interestingly, adult $R26R^{YFP/+}$UBC-$CreT2^{+}$ mice weaned prior to the initiation treatment responded very differently and remained YFP-negative despite direct ingestion of ivermectin containing feed (data not shown). Neither the $R26R^{YFP}$ knock-in allele nor the UBC-CreT2 transgene alone was sufficient for YFP expression induced by ivermectin (data not shown).

To determine if CreT2-mediated deletion induced by ivermectin occurred in all tissues or only a subset, histologic sections of thymus, spleen, and kidney from $R26R^{YFP/+}$UBC-$CreT2^{+}$, $R26R^{YFP/+}$UBC-$CreT2^{-}$ and tamoxifen-treated mice were evaluated for YFP expression. Oral tamoxifen treatment resulted in YFP expression regardless of cell or tissue type (data not shown). In contrast, ivermectin treatment resulted in limited distribution of YFP expression, with YFP detected in spleen and thymus, but not kidney tissue in ivermectin-treated experimental mice (preliminary data not shown) suggesting potential hematopoietic specificity. Systematic examination of splenic populations using flow cytometry confirmed that YFP was expressed by up to 80% of $CD4^{+}$ and $CD8^{+}$ T cells, but less than one percent of B lymphocytes (Fig. 1b).

Because deletion was relatively restricted to T cells, we further evaluated thymocytes of ivermectin treated mice to determine when deletion occurred during T cell development. Development appeared grossly normal in all mice (data not shown). YFP was first detectable between the $CD4^{-}CD8^{-}CD25^{+}CD44^{-}$ (double negative 3, DN3) and $CD4^{+}CD8^{+}$ (double positive, DP) stages of T cell development (Fig. 1c).

We propose the following potential mechanism to explain the findings presented above. Since deletion occurs in offspring prior to weaning and not in genotypically identical ivermectin treated adults, we believe that metabolites may be transmitted transplacentally and/or via lactation. Secretion of ivermectin correlates with fat content of the milk[10]. It is possible that fat stores within the mother are acting as a reservoir, explaining how deletion could occur long after ivermectin treatment had been stopped. Regardless of underlying mechanism, the persistent finding of CreT2-mediated deletion in offspring born from distantly treated breeders resulted in the time-consuming need to generate new breeder pairs.

We report these findings to inform the community of this unintended effect of ivermectin treatment. The consequences of ivermectin-induced deletion, while confined to offspring rather than previously weaned mice, render both breeders and offspring unusable for future experiments. Breeding cages using parents who were free of ivermectin from birth subsequently produced offspring without evidence of tamoxifen-independent deletion. Thus, at minimum twenty-two weeks is required to generate mature (six to eight week old) experimental mice after treatment has ended. Because of the long-term effects of oral ivermectin on mice using tamoxifen-induced temporal deletion, caution should be applied before beginning treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

1. Weisbroth SH, et al. Lab. Anim. Sci. 1974; 24:510–516. [PubMed: 4365340]
2. Weisbroth SH, et al. Lab. Anim. Sci. 1976; 26:725–735. [PubMed: 979139]
3. Dourmishev A, et al. International Journal of Dermatology. 2005; 44:981–988. [PubMed: 16409259]
4. Blakley BR, Rousseaux CG. Am. J. Vet. Res. 1991; 52:593–595. [PubMed: 1828942]
5. Edwards G. Filaria J. 2003; 2(Suppl 1):S8. [PubMed: 14975065]
6. Srinivas S, et al. BMC Developmental Biology. 2001; 1:4. [PubMed: 11299042]
7. Ruzankina Y, et al. Cell Stem Cell. 2007; 1:113–126. [PubMed: 18371340]
8. Feil R, et al. Biochemical and Biophysical Research Communications. 1997; 237:752–757. [PubMed: 9299439]
9. Wu GF, et al. Eur. J. Immunol. 2011; 41:2064–2073. [PubMed: 21469089]
10. González Canga A, et al. The Veterinary Journal. 2009; 179:25–37. [PubMed: 17851096]

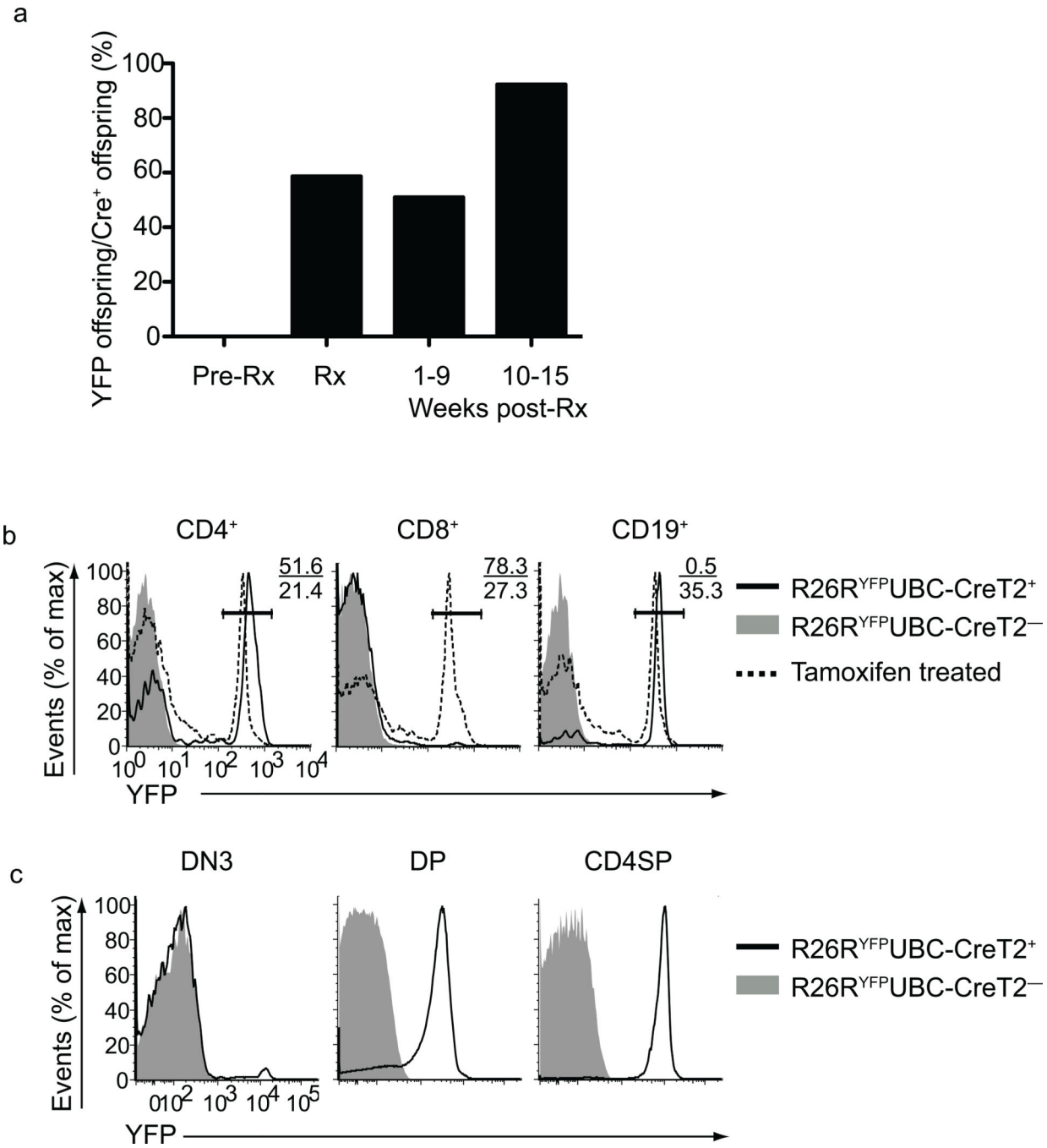

Figure 1. Tamoxifen-independent CreT2-mediated deletion

A: Heparinized peripheral blood from R26R$^{YFP/+}$UBC-CreT2^{+} offspring was assessed for YFP expression by flow cytometry. The relative percentage of offspring with cells expressing YFP before, during, and after completion of ivermectin treatment (R_x) is shown. Data are derived from two hundred eighty-seven individual mice from over seventy-five litters in thirty-three breeding cages. **B:** Splenocytes from three week old R26R$^{YFP/+}$UBC-CreT2^{+} (black line), R26R$^{YFP/+}$UBC-CreT2^{-} (shaded), and tamoxifen-treated (dashed line) mice were stained with CD4, CD8, and CD19. Histograms showing relative intensity of

YFP are gated on $CD4^+$ T (Left), $CD8^+$ T (Center) or $CD19^+$ B (Right) cells. Relative percentage of cells within the gated area are indicated for $R26R^{YFP/+}$UBC-$CreT2^+$ (top), $R26R^{YFP/+}$UBC-$CreT2^-$ (bottom) within each plot. Data are representative of two experiments with greater then three mice each. **C:** Thymocytes from $R26R^{YFP/+}$UBC-$CreT2^+$ (black line) and $R26R^{YFP/+}$UBC-$CreT2^-$ (shaded) littermates were stained with CD4, CD8, CD25, cKit and a lineage cocktail. Histograms are gated on DN3 (CD4-, CD8-, lineage-, ckit-, CD25+), DP (CD4+CD8+), or CD4SP populations. Data are representative of two experiments with three or more mice.