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LETTER TO THE EDITOR

The influence of vaginally applied imiquimod on the course of *Chlamydia trachomatis* serovar D infection in a murine model

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Sir—We read with great interest the article by Ramsey and colleagues [1]. In their study, they concluded that imiquimod had no efficacy in controlling *Chlamydia trachomatis* MoPn (mouse pneumonitis agent) infection in a murine model of female genital tract infection. Imiquimod is an immune response modifier that induces the production of an array of essentially Th1 response promoting cytokines, most notably tumor necrosis factor-alpha and interferon-gamma, the pluripotent cytokine that is considered essential in both the innate and the acquired immune response to *C. trachomatis* genital tract infection.

We, as well as others, have shown that the course of female genital tract infection with MoPn in IFN-gamma knockout mice is only minimally different when compared with genetically intact mice, whereas infection with a human isolate of *C. trachomatis* serovar D is uncontrolled during the acute phase and of significantly longer duration in interferon-gamma deficient mice [2, 3]. Therefore, we investigated the effect of various regimens of vaginally applied imiquimod on the course of infection in essentially the same murine model of *C. trachomatis* female genital tract infection as used by Ramsey and colleagues, but employing a strain belonging to the human oculogenital biovar of *C. trachomatis*.

In brief, CF-1 mice treated with progesterone were inoculated intravaginally by direct instillation of 10 μl of bacterial suspension containing 1 X 10^5 inclusion-forming units (ifu) of *C. trachomatis* serovar D [4]. On the days before (−) and after (+) infection as indicated in the results table, 10 μl of imiquimod (Aldara^{TM}), diluted 1:4 in saline or a placebo similar in composition to the inactive base used in the imiquimod preparation, was administered intravaginally [5]. The presence of *Chlamydia* in the lower genital tract was determined by culturing the vaginal contents as previously described, and the duration of infection between groups was analyzed using the Wilcoxon rank sum test [2, 4, 5].

As displayed below, we observed a statistically significant reduction in the median duration of infection in mice treated prophylactically with imiquimod several times before and during the acute phase of *C. trachomatis* infection, i.e. 4 days for treated animals compared with 19 days for the group receiving placebo. No effect on the course of infection was seen with other regimens, a single application 2 h before or multiple applications every other day beginning 4 days after chlamydial inoculation and ending on day 14 (data not shown).

Remarkably, in the prophylactically treated group on day 2, no effect was observed on the incidence of infection when compared with the placebo group. However, by day 4 differences between the groups were apparent, implying that treated mice were primed to respond more rapidly to infection, while apparently unaltered in their susceptibility to initial infection. In mice, imiquimod is reported to act through the Toll-like receptor 7 (TLR7) MyD88-dependent signalling pathway This suggests that, in contrast to the speculation of Ramsey and colleagues, sufficient quantities of the TLR7 are expressed in the mouse genital epithelium or in tissues adjacent to the genital epithelium to allow a response to imiquimod.

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Table 1 The Influence of a Multiple Intravaginal Application Regimen of Imiquimod on the Course of *Chlamydia trachomatis* Serovar D Genital Tract Infection

Treatment Group	Animal	Culture Results on Indicated Day Post-Infection																Duration	Median Duration	Rank Sum p Value
		2	4	7	10	14	17	21	24	28	35	42	49	53	60	63	67			
	1	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4		
	2	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	17		
	3	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4		
Imiquimod	4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4	< 0.05
-5,- 3, -1	5	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2		
+ 1, + 3, + 5	6	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	10		
	7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	28		
	8	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4		
	1	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	63		
Base	2	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	7		
-5,- 3, -1	3	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	28	19	–
+ 1, + 3, + 5	4	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	10		
	1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2		
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	53		
	3	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	28		
None	4	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	10	28	*
	5	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	28		
	6	+	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	42		
	7	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	10		
	8	+	+	+	+	-	-	-	+	-	+	+	-	-	-	-	-	42		

*Not different from Base Control Group

The significant difference between our results and the results of Ramsey and colleagues may be a result of the genetic background of the mouse strains involveded (BALB/c versus CF-1), the slightly different prophylactic regimens tested, or, as our previous studies suggest, the strain of *C. trachomatis* used in the setting of the murine female genital tract (MoPn versus human urogenital isolates such as serovar D). With regard to the latter, and based on published differences between these two biovars of *Chlamydia*, we have recently suggested that the use of human urogenital isolates may be more appropriate in certain types of experiments in which these differences could affect the translational value of the results obtained [6].

In conclusion, in the much used murine model of the human female genital tract infection and in contrast to the results of Ramsey and colleagues, we observed that imiquimod intravaginally applied prophylactically on multiple occasions, days before infection, may enhance local natural immunity and shorten the duration of genital tract infection with a human isolate of *C. trachomatis*, perhaps through a mechanism of enhanced local natural immunity. Understanding the mechanism of this enhanced responsiveness might provide insight into the complex immunobiology of female genital tract infection with *C. trachomatis* and may lead to new prevention and treatment methods. In this context, it is interesting to speculate if women using the multiple application regimen of imiquimod approved for the treatment of genital warts might realize an alteration in the cytokine responsiveness within the genital tract and a possible reduced risk of *C. trachomatis* genital tract infection.

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