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IDENTIFICATION OF CANDIDATE GENES USING THE MURINE MODEL OF FEMALE GENITAL TRACT INFECTION WITH *CHLAMYDIA TRACHOMATIS*

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SUMMARY

The integrated approach to the study of female genital tract infection (GTI) with Chlamydia trachomatis *is a conceptual framework through which a consistent and comprehensive evidence-based understanding of* C. trachomatis *GTI could evolve. One application of this approach has been to identify candidate genes that may play a role in the course and severity of* C. trachomatis *GTI in women, using human clinical and genetic data together with results obtained in the female mouse model to guide the selection process. This model has been proven robust enough: i) to identify stable phenotypic differences in the course and outcome of GTI among commonly used immunocompetent inbred mouse stains that are used in the construction of gene knockout (KO) and transgenic mice; as well as ii) to serve as a platform in which to assess the influence of genetic differences among human genital tract isolates of* C. trachomatis *as well as between this biovar and the mouse biovar,* Chlamydia muridarum. *This review presents a summary of published and unpublished results from 25 years of studies in immunodeficient and gene-deficient KO mice that both inform our present understanding of the immunogenetics of* C. trachomatis *GTI and serve to guide candidate gene selection.*

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BACKGROUND

For over a decade, we have advocated an integrated approach to the study of female genital tract infection with *Chlamydia trachomatis* that draws from: i) human immunogenetic, epidemiologic and clinical data; ii) in vitro analysis of the extracellular interactions and intracellular molecular events that occur during chlamydial infection; and iii) the course and outcome of genital tract infection with *C. trachomatis* in animal models of disease. Essential to the value of the latter is the degree to which a model of disease mimics the natural course and outcome of infection in women and allows for the assessment of the complex interplay between the diverse immune and inflammatory elements of the host response to infection at both the cellular and molecular levels.

Perhaps the best defined and most used model of infection and host response to chlamydiae is the female mouse genital tract infection system using the murine isolate, *C. muridarum* (the mouse pneumonitis agent, MoPn) (1, 2), or human isolates of *C. trachomatis* (serovars D-K, L1-L3) (3, 4). The murine model exhibits vital aspects that mimic human genital chlamydial infection including: the shedding of viable elementary bodies during the course of an active infection, and the observed pathologic consequences or sequelae of infection, ranging from mild lower genital tract-limited inflammation to tubal inflammation, hydrosalpinx formation and infertility. Infection with MoPn results in the latter and is considered a model of the more severe upper genital tract sequelae associated with ascending *C. trachomatis* infection in women, i.e., pelvic inflammatory disease (PID), ectopic pregnancy and infertility (5, 6). Infection of the female mouse genital tract with the human urogenital biovar of *C. trachomatis* (serovars D-K) is limited in the ability to ascend from the initial site of infection within the lower genital tract (4, 7), and is thus a model of the vast majority of human infections caused by this biovar.

In addition, the immune responses following an infection and the immune parameters for controlling chlamydiae in mice, and the acquisition of temporary resistance (protective immunity) against reinfection, are similar in both animal species. Moreover, although the mouse does not correspond to the human system in all respects, the innate and adaptive immune systems and their molecular and cellular effectors are comparable. Thus, in most instances where parallels have been drawn, there is every reason to think that data collected in the mouse model often will reflect immune parameters important in human disease (2, 8).

Numerous laboratories that utilize the mouse model of chlamydial genital disease have established that immunocompetent mice vaginally infected with either *C. muridarum* or strains of *C. trachomatis* undergo a course of infection marked by shedding of chlamydiae into the vaginal vault for up to 2–6 weeks, depending on the inoculum and chlamydial strain (4, 9). A temporary, 60–90-day protective immunity against reinfection is established following the resolution of infection in a previously naïve animal. Reinfection during this period results in a lower level of shedding and a shorter duration of infection that usually resolves within 3 weeks (10, 11). Although specific B and CD4 and CD8 T-cell subsets as well as specific antichlamydial antibodies comprising all isotypes are induced following a primary infection (2, 10, 12), protective chlamydial immunity correlates with a strong CD4 T helper type 1 (Th1) response (13). Noteworthy in this regard was the demonstration that the elusive but important role of antibodies in chlamydial immunity is associated with the ability to enhance chlamydial antigen uptake and presentation for a rapid and robust T-cell response (14, 15).

It is in this context that the role of host genetics in chlamydial infection and disease processes has evolved along with an appreciation that the murine model has been instrumental in suggesting this role, and will continue to be an indispensable tool to assess the role of specific genes in the course and outcome of chlamydial infection of the female genital tract. Most of the commonly used immunocompetent inbred strains of mice (BALB/c, C57BL/6 and C3H/HeJ) have been utilized and characterized in the model (9, 10), and each has been shown to express a uniquely characteristic set of phenotypic responses to infection that includes: the level, pattern and duration of infection; the quantitative and qualitative cellular and humoral immune responses induced by infection; the nature and degree of the adaptive immunity provided against reinfection; and the cytokine, chemokine and signaling pathways that participate in the complex response to infection. These phenotypic differences have made them useful as a means of suggesting genetically determined factors that may play a role in the course and outcome of infection, and have guided the selection of genes that when inactivated might alter the susceptibility to and course of infection.

The purpose of this review will be to present a summary of results that have utilized immunodeficient and gene-

deficient knockout (KO) mice in the murine model. Also, the results obtained using the human biovar and those that used the mouse biovar will be presented separately because of the significant differences in the course and pathological outcome repeatedly observed between infection with these two species of *Chlamydia*. Published and unpublished results from 25 years of studies will be tabulated in an effort to provide the starting point for the next cycle of candidate gene selection.

THE MURINE MODEL

The following description of the murine model contains all the salient elements that are currently included by the vast majority of investigators who use the model, including all of the studies cited in this review.

In order to induce a prolonged diestrous-like state within the genital tract and thus enhance the infection rate and duration following a single intravaginal challenge, progesterone in the form of medroxyprogesterone acetate is administered subcutaneously in 2.5 mg doses, either 1 or 2 times between 10 and 3 days prior to infection. Mice are inoculated intravaginally with 10–50 µL of an elementary body suspension containing 10^4–10^7 inclusion forming units (ifu) of *C. trachomatis* strains of the human biovar (usually serovars D or E) or 10^3–10^7 ifu of MoPn. The presence of *Chlamydia* in the lower genital tract is determined by swabbing the vaginal vault and ectocervix with a calcium alginate or Dacron swab, and the bacterial content is quantified using standard culture, polymerase chain reaction (PCR), or direct detection methods. Blood and vaginal contents are periodically obtained for serologic and cytokine/chemokine analysis. The course of infection is routinely monitored for 2 months and the gross pathologic outcome is assessed visually following euthanasia, at which time lymphoid tissues for in vitro determination of cellular immune status, and genital tract tissues for histopathologic evaluation are collected.

The robustness of the model to characterize a number of measurable features of the course of infection, such as the level and pattern of shedding and the duration of infection, and to identify significant differences in these features between strains of two commonly used and immunogenetically well characterized strains of mice is captured in Table I, which shows the cultures results obtained following initial and subsequent female genital tract infection of BALB/c and C57BL/6 mice with a serovar D strain of *C. trachomatis*.

In addition to providing insight into the possible role of innate and acquired immune pathways on the course of infection and disease progression, the significant differences observed between these strains of mice also highlight the need to be mindful of stable genetically determined background differences that influence the outcome of infection when assigning a cellular and molecular influence of a particular gene of interest. Of particular significance with regard to this review is the fact that many gene knockout mice are backcrossed into these two well-characterized inbred strains resulting in mixed pedigrees, which raises the obvious concern about the possible impact that even slight pedigree differences between the knockout and genetically intact control mice might have on the ability to assign the exact genetic cause of any observed difference with certainty.

DISCUSSION

To date, we and others have utilized the mouse model to study the role of numerous infection-modifying factors in both genetically intact immunodeficient and knockout mouse strains. Table II summarizes the results of these studies.

Because these studies span 2 decades, the pedigree of the mice was not always stated or clearly defined and for that reason no reference is made to this perhaps significant variable in assigning an influence of a particular gene on the course and outcome of infection. Rather, the investigator's assignment of cause for any observed differences between the gene-deficient mice and the genetically intact mice used as the control have been accepted and are simply reported in a summary form in this review. For specific details, the reader is referred to the original publications or the individual investigators in the case of the unpublished results that are cited with permission. Additionally and because of the biased use of MoPn over this period, most of the genes studied in KO mice have yet to be repeated using strains of *C. trachomatis* isolated from humans.

However and with the notable exception of the possible difference in the role of interferon (IFN)-γ following infection with either biovar, the essential role of T cells in all aspects of genital tract infection and progression has been confirmed, beginning with studies in athymic mice to the most recent studies in gene knockout mice that have assessed the role of key mediators of T-cell activity and inflammation to infection and/or antigen reactivity. In addition, these results clearly demonstrate the roles of IFN-γ and interleukin-12 in both the control

Table I. *Comparison of initial and homotypic reinfection with* C. trachomatis *serovar D in C57BL/6 and BALB/C mice (10).*

Mouse strain	Group	Animal	Initial infection duration	Number of animals culture positive on indicated day after infection Number of inclusion forming units (ifu) for primary culture positive specimens 2	4	7	10	14	17	21	24	28	31	35	38	42	45	50	56	59	63	66	70	Infection duration	Infection duration Median	Wilcoxon rank sum P-value
C57BL/6	Reinfected	64-1	7	390	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4	0.05
		64-2	31	490	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	24		
		65-3	46	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4		
		65-4	31	770	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4		
		66-3	17	260	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	+	-	66		
		66-4	56	250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2		
			Ave. ifu	432	20	0	0	0																		
	Control	67-1	NA	9,210	1,760	390	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	40	
		67-3	NA	3,800	1,410	60	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	28		
		68-2	NA	7,560	3,080	40	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	50		
		68-3	NA	4,620	100	10	10	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	45		
		69-2	NA	2,840	570	460	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	59		
		69-3	NA	3,400	290	60	20	20	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	35		
			Ave. ifu	5,238	1,202	170	15	15																		
BALB/C	Reinfected	84-1	7	3,940	+	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	5.5	0.02
		84-3	28	630	20	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7		
		85-3	7	19,940	40	20	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10		
		85-4	21	8,250	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2		
		86-3	10	610	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2		
		86-4	10	1,590	240	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4		
			Ave. ifu	5,827	100	30	100	0																		
	Control	87-1	NA	11,550	28,710	8,250	3,630	10	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	28	12	
		87-2	NA	44,140	8,690	2,520	360	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10		
		88-1	NA	141,900	50,780	1,860	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7		
		88-2	NA	32,450	22,440	200	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	24		
		89-1	NA	27,090	14,410	1,510	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10		
		89-4	NA	26,290	9,570	100	550	310	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14		
			Ave. ifu	47,237	22,433	2,407	1,148	160																		

NA, not available

Table II. *Summary of the course and outcome of chlamydial genital tract infection in immunogenetically deficient and knockout mouse strains (11).*

		Outcome within the genital tract following infection with the indicated *Chlamydia* biovar		
Strain	Altered function(s)	Human	Mouse	Ref(s)
Nude	T-cell immunity	More shedding, infection does not resolve, severe pathology	Infection does not resolve	16, 17
SCID	T- and B-cell immunity	Not done	Infection does not resolve	18
CD4	CD4 T-cell functions	Not done	Delayed resolution	19
β2 microglobulin	CD8 T-cell functions	Not done	No effect	19
T-cell receptor β chain	αβ T-cell functions	Not done	Infection does not resolve	19
T-cell receptor γ chain	γδ T-cell functions	Not done	No effect	19
MHC class II	MHC class II restricted immunity	More shedding	Infection does not resolve	19, 20
Immunoglobulin micro chain (μMT)	B cell and antibody functions	No effect on primary infection; reduced susceptibility to reinfection	No effect on primary infection; increased susceptibility to reinfection	21-23
Fc γ receptors I, II, & III	Inflammatory responses, phagocytosis, antigen presentation and ADCC	Not done	No effect on primary infection; more shedding, longer duration, more severe pathology on reinfection	24
Interferon-γ	Multiple inflammatory and immune responses	More shedding, infection does not resolve, severe pathology	More shedding, delayed resolution, systemic dissemination	19, 25, 26
Interferon-γ receptor	Multiple inflammatory and immune responses mediated by IFN-γ	More shedding, delayed resolution, severe pathology	Delayed resolution	25, 26
IL-12	NK and CD-8 T-cell activation, Th1 immune responses	No effect on shedding, persistent infection (9) More shedding (14)	More shedding, longer duration	25, 27
IL-6	Acute inflammation	No effect	No effect	27, 28
TNF-α p55 receptor	TNF-α inflammatory and immune functions mediated through TNF-α p55 receptor	More shedding, longer duration (14) No effect (9)	More shedding, longer duration	29
TNF-α p55 & p75 receptors	TNF-α inflammatory and immune functions mediated through TNF-α p55 & p75 receptors	No effect on primary infection; increased susceptibility to reinfection	Not done	29
iNOS	Intracellular killing, NK cell activation	More shedding, longer duration	Persistent infection, increased incidence of hydrosalpinx	30-32
Phagocyte NADPH oxidase	Reactive oxygen species functions	Not done	No effect on infection, less pathology	23
Nramp-1	Intracellular killing, antigen presentation, NOS induction	Not done	No effect	33

Continued

Table II. *Summary of the course and outcome of chlamydial genital tract infection in immunogenetically deficient and knockout mouse strains (11). (Continued)*

		Outcome within the genital tract following infection with the indicated *Chlamydia* biovar		
Strain	Altered function(s)	Human	Mouse	Ref(s)
ICAM -1	Inflammation, T-cell recruitment, antigen presentation	Not done	More shedding, upper tract infection, longer duration; severe pathology after multiple infections	34
Fas	CD4 and CD8 T-cell mediated apoptosis	Not done	No effect	35
Fas ligand	CD4 and CD8 T-cell mediated apoptosis	Not done	No effect	35
Perforin	CD8 T-cell mediated cytotoxicity	Not done	No effect	35
Perforin & Fas ligand	CD4 and CD8 mediated apoptosis and CD8 mediated cytotoxicity	Not done	No effect	35
Bax	Apoptosis via mitochondrial membrane disruption	Not done	Decreased shedding, shorter duration, granuloma formation	36
Toll-like receptor (TLR) -2	Pathogen recognition receptor responses (PRRR) via peptidoglycan, lipo- and glycoproteins	No effect on susceptibility. Slight effect on symptomatology and severity (44)	No effect on infection, less pathology	37, 38
TLR -4	PRRR via LPS and HSP60	No effect on susceptibility. Slight effect on severity (45)	Less protection upon reinfection	37, 38
TLR -9	PRRR via unmethylated CpG DNA	Slight effect on susceptibility and severity (46)	Slight protection upon reinfection	38
Nod1	PRRR via peptidoglycan	No effect	No effect	39
Rip2	PRR driven inflammatory and immune responses	Not done	Small increase in shedding, duration and pathology	39
MyD88	PRR driven inflammatory and immune responses	Not done	Slower resolution	40
Matrix metalloproteinase-7	α-defensin activation	Not done	No effect	41
Chemokine receptor CCR5	T-cell activation	Protection against tubal pathology	More shedding, slower resolution, less severe pathology	42

MHC, major histocompatibility complex; TNF, tumor necrosis factor; NADPH, nicotinamide adenine dinucleotide phosphate; ICAM, intercellular adhesion molecule; ADCC, antibody-dependent cell-mediated cytotoxicity; IFN, interferon; NK, natural killer; NOS, nitric oxide symthase; LPS, lipopolysaccharide; HSP, heat shock protein.

of infection and the induction of protective acquired immunity, and in the case IFN-γ a role in the progression to severe upper genital tract pathology following infection with human isolates.

Of noteworthy significance, is that interleukin-6, a well-characterized mediator of inflammation, had no observed effect during initial infection or on the level of acquired immunity following resolution, while the absence of tumor necrosis factor-α receptors resulted in the altered induction of both innate and adaptive immune responses to infection. Finally, no significant effect on the course of infection or induction of acquired immunity has been observed in any single pathogen recognition receptor (PRR) response KO mouse strain tested to date. However, the absence of PRRs in the signal transduction pathways through which these PRR responses operate may reduce the level of innate immune activation following infection.

CONCLUSIONS

One aim of the integrated approach to the study of female genital tract infection with *C. trachomatis* has been to identify candidate genes that may play a role in the susceptibility to and severity of *C. trachomatis* infection in women, using human immunogenetic data from other infectious diseases and results obtained in the murine model to guide the selection process. The results obtained over the past 25 years using the murine model have demonstrated that parallels exist in the disease processes and immune responses observed in women and female mice infected with chlamydiae, and, more importantly, these similarities can be used to identify and assess specific genes that may play a role in human infections with this agent.

With the possible exception of IFN-γ in the mouse, no single inflammation- or immunity-modifying gene has been shown to singularly determine the susceptibility to infection or the severity of the outcome observed in either women with *C. trachomatis* genital tract infection or in the murine model. However, women with mutations in multiple PRR genes are at greater risk of tubal pathology-associated infertility (43), which would be predicted by the results obtained in mice. Thus, appropriately defining multigene traits based on even small observed effects on infection in KO mice might be one approach to enhance the translational value of the murine model in candidate gene selection. By creating more sophisticated, conditional and tissue-specific KO mice, we will be able to manipulate and better mimic dynamic processes in which the gene of interest might play a role. Based on the findings to date, there is every reason to believe that the murine model will continue to be a valuable tool that provides translationally useful knowledge (44-46).

Finally, the diverse spectrum of infection and pathological outcomes observed during female genital tract infection with *C. trachomatis* is likely the result of the eons long co-evolutionary dance between the human female genital tract and the collection of strains that comprise the oculogenital biovar of *C. trachomatis*. For this reason and in order to understand the infection altering phenotypic differences between *C. muridarum* and *C. trachomatis* that have been described, we are of the strong opinion that it is necessary to simultaneously assess the role of regulatory elements of the immune and inflammatory responses in the female mouse genital tract during both the rapidly progressive and tissue destructive processes that occur during *C. muridarum* infection and the more subtle and limited course of infection with human isolates of *C. trachomatis*.

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DISCLOSURE

The authors have nothing to disclose.

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