added a fourfold excess of probe from a freshly prepared concentrated dimethylformamide solution. The reaction was kept on ice for 30 min before extensive dialysis in 20 mM Hepes (pH 7.2), 0.16 M NaCl, 1 mM DTT, and 2 mM EGTA. Peptides were labeled with DABMI (4-dimethylaminophenylazophenyl-4′-maleimide), a nonfluorescent energy transfer acceptor, in the presence of an equimolar amount of dye in the absence of reducing agents. The reaction was allowed to proceed for 30 min at room temperature and quenched with 5 mM DTT. The final labeling ratios, as determined by absorption, were 1:1 for Cascade Blue–PLC-β2 and 0.8 for the two DABMI peptides. Fluorescence spectra were taken on an ISS-PC1 (ISS, Champaign, IL) photon-counting spectrofluorometer in a 3 mm by 3 mm cuvette with excitation at 380 nM and scanning from 400 to 560 nM. The FRET experiment was done under the same solution conditions as the PLC assay and the labeled peptide could still activate PLC. In fluorescent studies done in the presence of membranes, the lipid concentration was high enough so that all PLC was membrane-bound (*14*).

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Chlamydia Infections and Heart Disease Linked Through Antigenic Mimicry

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Chlamydia infections are epidemiologically linked to human heart disease. A peptide from the murine heart muscle–specific α myosin heavy chain that has sequence homology to the 60-kilodalton cysteine-rich outer membrane proteins of *Chlamydia pneumoniae*, *C. psittaci*, and *C. trachomatis* was shown to induce autoimmune inflammatory heart disease in mice. Injection of the homologous *Chlamydia* peptides into mice also induced perivascular inflammation, fibrotic changes, and blood vessel occlusion in the heart, as well as triggering T and B cell reactivity to the homologous endogenous heart muscle–specific peptide. *Chlamydia* DNA functioned as an adjuvant in the triggering of peptide-induced inflammatory heart disease. Infection with *C. trachomatis* led to the production of autoantibodies to heart muscle–specific epitopes. Thus, *Chlamydia*-mediated heart disease is induced by antigenic mimicry of a heart muscle–specific protein.

Cardiovascular diseases are the major cause of death in Western societies. Various risk factors have been associated with the pathogenesis of heart diseases, including increased cholesterol levels, smoking, stress, high blood pressure, obesity, and hyperglycemia (*1*). Bacterial infections may be a causative event in the development of heart diseases (*2*, *3*). *Chlamydia* infections cause pneumonia, conjunctivitis in children, and are a primary cause of sexually transmitted diseases and female infertility (*4*). The mechanism by which *Chlamydia* causes cardiovascular disease is unknown (*5*).

Inflammatory heart diseases and dilated cardiomyopathy in humans can be reproduced in mice by immunization with heart muscle myosin (*6*). Cardiac myosin–induced autoimmune myocarditis is dependent on $CD4^+$ T cells that recognize a heart muscle–specific peptide in association with self major histocompatibilty complex (MHC) class II molecules (*7*). Various peptides of the α myosin heavy chain protein have been identified that can induce autoimmune myocarditis in mice (*8*, *9*).

Immunization with a 30–amino acid peptide (amino acids 614 to 643) of the cardiac-specific α myosin heavy chain molecule [αmhc(614–643)] induces severe inflammatory heart disease in BALB/c mice (*8*). The first 16 amino acids [αmhc(614–629), SLKLMATLFSTYASAD] constituted a dominant autoaggressive epitope that was designated M7Aα (Table 1 and Fig. 1A) (*10*). In contrast, the homologous region of the β myosin heavy chain isoform, designated M7Aβ, did not induce disease (Table 1 and Fig. 1B). The introduction of single amino acid substitutions into M7Aα further revealed that the residues xxx-MAxxxSTxxx (where x is any amino acid) were important for the pathogenicity of M7Aα in vivo (*11*). These immunogenic amino acids are conserved between murine and human α myosin heavy chains, and injection of the human M7Aα homolog into BALB/c mice also induced inflammatory heart disease (*11*).

After identification of the crucial pathogenic amino acids within the M7Aα peptide, we screened public databases for viral and bacterial sequences containing the MAxxxST motif (*12*). Peptide sequences from the 60-kD cysteine-rich outer membrane protein (CRP) from different serovars of *C. trachomatis* matched the M7Aα motif and were designated ChTR1 (serovar E), ChTR2 (serovar C), and ChTR3 (serovars L1, L2, and L3) (*13*).

Table 1. Sequence alignment of *Chlamydia* peptides, the immunogenic mouse M7Aα motif, and the nonimmunogenic mouse M7Aβ motif. Prevalence and severity of inflammatory heart disease as determined with these peptides are indicated. Six-week-old BALB/c mice were immunized twice at a 7-day interval with the indicated peptides (50 μg per mouse] in FCA and analyzed 21 days after the initial immunization for the presence and severity of myocarditis. Histological grading of severity was as follows: 0, no infiltration in heart muscle; 1, up to 5% of histological cross section is infiltrated; 2, 6 to 10%; 3, 11 to 20%; 4, >20%. Mean values of disease severity ± SD are indicated (*6*, *10*).

Peptide	Amino Acid Sequence	Prevalence	(%)	Severity
M7Aα (614-629):	SLKLMATLF STYASAD	18/21	(86%)	2.9 ± 0.7
ChTR1 (25-40):	VLETSMAEFTSTNVIS	12/15	(80%)	1.4 ± 0.4
ChTR2 (25-40):	VLETSMAESLSTNVIS	7/8	(88%)	1.3 ± 0.5
ChTR3 (25-40):	VLETSMAEFI STNVIS	7/8	(88%)	1.1 ± 0.6
ChPN (25-40):	GIEAAVAESL I TKIVA	6/10	(60%)	1.1 ± 0.2
ChPS (25-40):	KIEAAAAESLATRFIA	5/10	(50%)	1.0 ± 0.0
ChTR p11 (1-14):	MGSMAFHKSRLFLT	4/8	(50%)	1.0 ± 0.0
M7Aβ (614-629):	SLKL LS NLFANYASAD	0/19	(0%)	

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The homologous peptides from the 60-kD CRPs of *C. pneumoniae*, designated ChPN, and *C. psittaci*, designated ChPS, shared sequence identities with the M7Aα motif, although to a lesser extent (Table 1) (*14*). Apart from identity at the MAxxxST motif, there were no other conserved regions in the primary sequences of the murine M7Aα peptide and all three *Chlamydia* 60-kD CRP peptides. A peptide from the p11 protein of *C. trachomatis*, designated ChTRp11, also shared sequence homology with the M7Aα motif (Table 1) (*15*).

We tested the possibility of antigenic mimicry between *Chlamydia* peptides and the M7Aα motif in our murine model of antigen-induced inflammatory heart disease. We immunized BALB/c mice with murine M7Aα or the homologous 60-kD CRP or p11-derived peptides in Freund's complete adjuvant (FCA) (*10*). All of the *Chlamydia*-derived peptides induced inflammatory heart disease at a similar frequency, although at significantly lower severity, as compared with M7Aα-immunized mice (Table 1). Like the inflammation that follows immunization with the endogenous autoantigen M7Aα, the disease induced by all the *Chlamydia*-derived peptides was characterized by perivascular and pericardial infiltration of mononuclear cells and fibrotic changes (Fig. 1, A, C, and D). Immunohistochemical characterization revealed that the inflammatory infiltrate in ChTR1 peptide–induced heart disease was similar to cardiac myosin– and cardiac myosin–derived peptide–induced myocarditis and consisted of about 11% $CD4^+$ and 12% $CD8^+$ T cells, 16% $B220^+$ B cells, and 61% $CD11b^+$ macrophages (*16*, *17*). Inflammation was restricted to the heart and was not observed in skeletal muscle, lung, liver, pancreas, kidney, intestine, or uterus of peptide-immunized mice. Injection of mice with human immunodeficiency virus–2 [gp160 (371–383), INFIGPGKGSDPE]– or parainfluenza virus 1 [HT83b hemagglutinin-neuraminidase (291–309), DLVFDILDLKGKTKSPRYK]–derived peptides that shared homology with other immunogenic regions of the mouse αmhc molecule [αmhc (735–747), GQFIDSGKGAEKL, and αmhc (314–332), DSAFDVLSFTAEEK-AGVYK] did not cause inflammatory heart disease (*8*, *11*). Thus, antigenic mimicry between *Chlamydia* peptides and a heart muscle–specific myosin peptide can lead to the development of inflammatory heart disease.

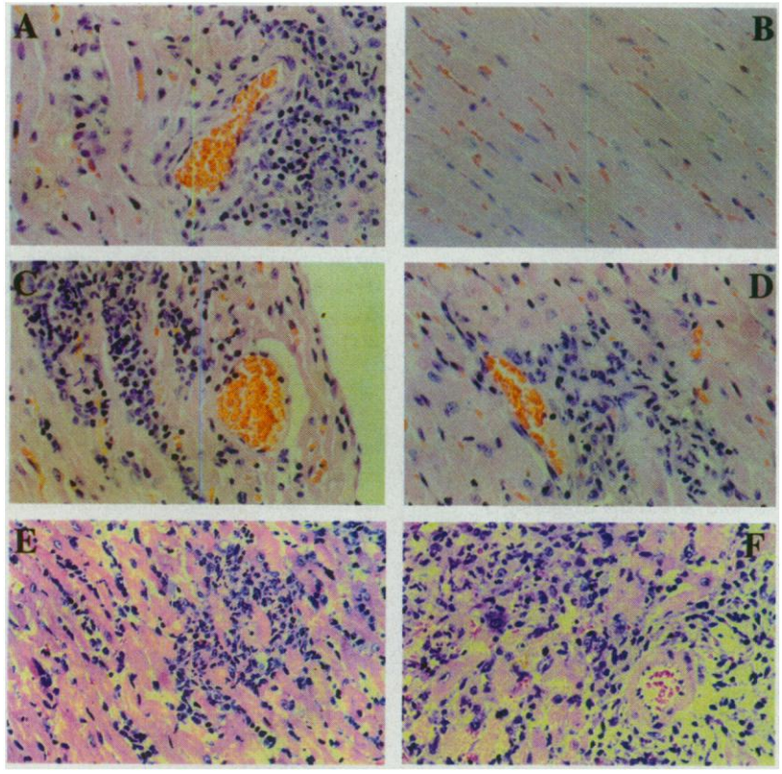


Fig. 1. Inflammatory heart disease in BALB/c mice that were immunized with **(A)** the endogenous mouse M7Aα peptide from the α myosin heavy chain, **(B)** the control endogenous M7Aβ peptide from the homologous region of the β myosin heavy chain, **(C)** the 60-kD CRP-derived peptide from *C. trachomatis* (ChTR1), or **(D)** the 60-kD CRP-derived peptide from *C. pneumoniae* (ChPN) (*10*). **(E)** Adoptive transfer of ChTR1 peptide–induced inflammatory heart disease into nonimmunized recipient mice (*21*). **(F)** Induction of inflammatory autoimmune heart disease in BALB/c mice with *C. trachomatis* DNA–derived CpG containing ODN as adjuvant (*25*). Perivascular inflammation is apparent in (A), (C), (D), and (F). (B) shows normal heart muscle morphology. Hearts were analyzed 21 days after the initial immunization. Staining was with hematoxylin and eosin (H&E). Magnification: ×320

Table 2. Prevalence and severity of M7Aα peptide–induced myocarditis as determined with synthetic ODNs or FCA as adjuvant. CpG motive–containing ODNs were derived either from *C. trachomatis* DNA (CpG 1) or from previously reported bacterial DNA sequences (CpG 2 and 3) (*25*). The CpG motif or the reversed non-CpG motif (non-CpG) is underlined. For severity of myocarditis, see Table 1. One result representative of three independent experiments is shown.

Adjuvant	Antigen	Prevalence	Severity
FCA	M7Aα	3/3	2.7 ± 1.5
CpG 1: GTACTGACGTTTACTCTTGG	M7Aα	5/5	2.2 ± 0.4
CpG 2: GATTGCCTGACGTCAGAGAG	M7Aα	4/4	2.3 ± 1.3
CpG 3: TCCATGACGTTCCTGACGTT	M7Aα	4/5	2.0 ± 1.4
Non-CpG: TCCATGAGCTTCCTGATGCT	M7Aα	0/5	–
CpG 3: TCCATGACGTTCCTGACGTT	None	0/5	–

The development of murine autoimmune myocarditis depends on the activation of $CD4^+$ T cells (*7*). To directly address the hypothesis of antigenic mimicry between an endogenous cardiac specific peptide and *Chlamydia*-derived peptides, we immunized BALB/c mice with M7Aα, ChTR1, or another cardiac-specific αmhc-derived peptide, designated kkα (*10*). This kkα peptide is restricted to $I\text{-}A^k$ MHC class II molecules, and kkα immunization induces myocarditis in A/J ($I\text{-}A^k$) mice (*9*) but not in BALB/c ($I\text{-}A^d$) mice (*11*). Immunization with M7Aα or ChTR1, but not with kkα or FCA alone, led to splenomegaly and large expansion of $TCR\alpha\beta^+$ $CD4^+$ T cells, $TCR\alpha\beta^+$ $CD8^+$ T cells, $B220^+$ B cells, and $CD11b^+$ macrophages, beginning 8 days after the initial immunization (*18*). Most (>50%) of $CD4^+$ and $CD8^+$ T cells expressed CD69 and CD25, indicating that these cells had been activated in vivo (*19*). Splenic T cells from mice immunized with the endogenous peptide M7Aα proliferated when incubated with splenocytes pulsed with the M7Aα peptide (Fig. 2A) (*20*). Splenic T cells from these mice also showed a strong proliferative response to the *C. trachomatis*–derived peptide ChTR1 (Fig. 2A). T cells from M7Aα- or ChTR1-immunized mice did not proliferate above control when incubated with γ-irradi-

ated splenocytes pulsed with the nonpathogenic kkα peptide. Splenic T cells from mice immunized with ChTR1 proliferated to ChTR1 and to the endogenous M7Aα peptide. Splenic T cells from control mice immunized with FCA only did not proliferate when activated with M7Aα. ChTR1, or kkα. Thus, ChTR1 peptide immunizations can cross-prime for T cell reactivity against the endogenous M7Aα.

Cardiac myosin–induced autoimmune myocarditis can be transferred adoptively into nonimmunized recipient mice (*7*). To establish the autoimmune basis of *Chlamydia* peptide–induced heart disease, we injected splenic T cells from ChTR1-immunized mice, restimulated in vitro with ChTR1 peptide and murine recombinant interleukin-2 (mrIL-2), into syngeneic BALB/c recipient mice (four mice per group) (*21*). All animals developed inflammatory heart disease similar (severity 1.0 ± 0.0) to that seen after direct immunization with ChTR1 peptide (Fig. 1E). Splenic T cells from FCA-immunized donors, stimulated in vitro with ChTR1 pepide and mrIL-2, did not induce myocarditis. Thus, ChTR1 peptide–induced myocardits can be transferred adoptively into nonimmunized recipient mice.

Murine autoimmune myocarditis is accompanied by the T cell–dependent production of autoantibodies to cardiac epitopes (*6*, *22*). Immunization with endogenous M7Aα peptide led to the production of serum antibodies to the M7Aα peptide used for the induction of the disease and to the ChTR1 peptide (Fig. 2B) (*23*). Likewise, immunization with the *C. trachomatis*–derived peptide ChTR1 induced the production of serum antibodies to ChTR1 and to the endogenous M7Aα peptide. Mice immunized with M7Aα or ChTR1 also produced antibodies to the kkα peptide (Fig. 2B), suggesting that M7Aα- and ChTR1-induced heart disease leads to epitope spreading at the B cell level.

How can *Chlamydia* infections in the lung or reproductive organs lead to the development of myocarditis? In our experimental model of inflammatory heart disease we used FCA as a potent immunoactivator. Bacterial DNA, but not mammalian DNA, has direct immunostimulatory effects in vitro and in vivo (*24*). We tested whether bacterial DNA–derived synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG islands could act as adjuvant for peptide-mediated autoimmunity. Various synthetic CpG motif–containing ODNs could trigger inflammatory autoimmune heart disease in M7Aα peptide–immunized BALB/c mice (Table 2 and Fig. 1F) (*25*). Immunization of BALB/c mice with a CpG ODN derived from the *C. trachomatis* CRP gene plus the M7Aα autoantigen induced inflammatory heart disease in the absence of FCA (Table 2 and Fig. 1F) (*25*). Immunizations in which a control

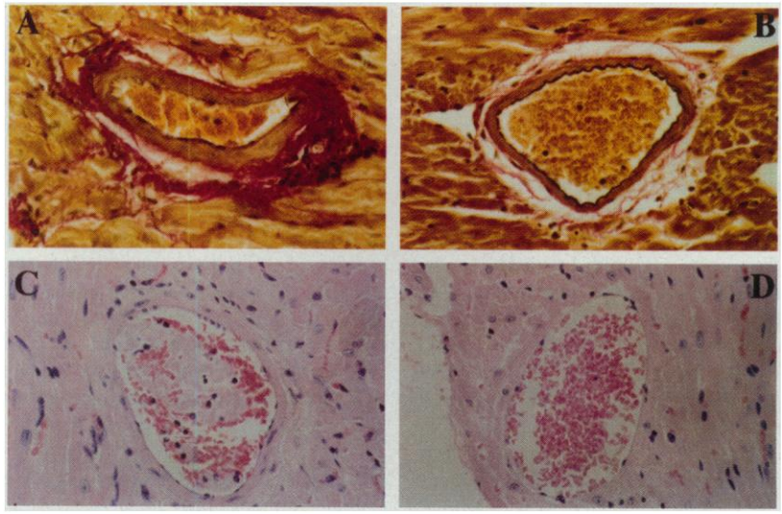
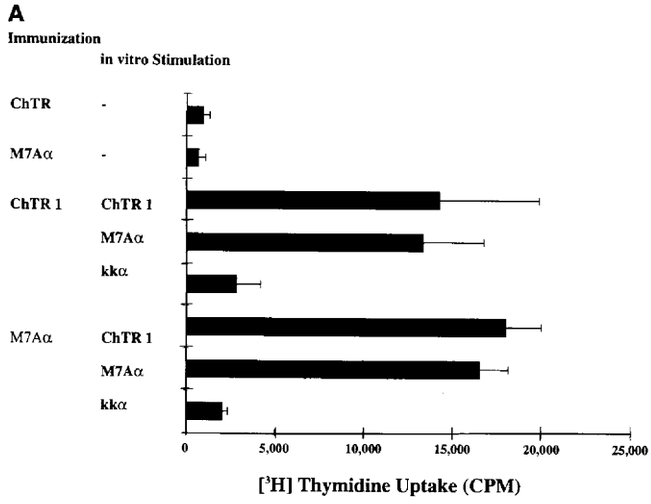


Fig. 3. Blood vessels in mice immunized with *C. trachomatis* 60-kD CRP-derived peptide (*10*, *30*). (**A**) Thickening of the arterial wall and perivascular fibrotic changes in mice immunized with ChTR1. The perivascular mononuclear inflammatory cells are apparent. (**B**) Normal morphology of the cardiac artery in mice immunized with FCA alone. (**C**) Occlusion of cardiac blood vessels in mice immunized with ChTR1. (**D**) No occlusions in cardiac blood vessels were seen in control mice immunized with FCA alone. (A and B) Elastica staining for collagen (red) to detect fibrotic changes. (C and D) H&E staining. Magnification, ×320.



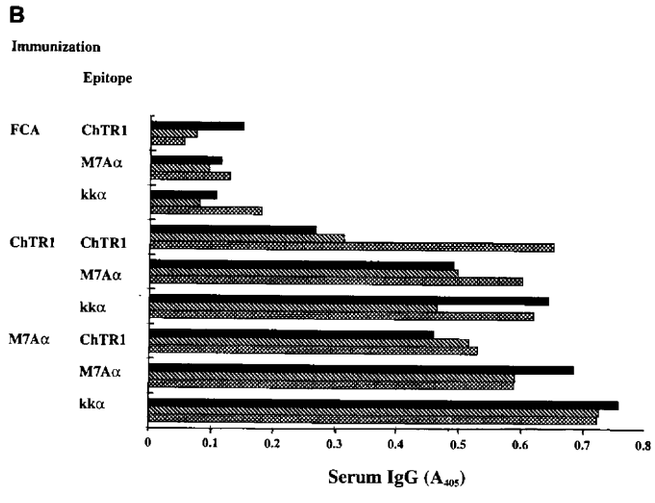


Fig. 2. (**A**) Splenic T cell proliferation and (**B**) serum IgG antibody production. (A) Proliferative responses to M7Aα, ChTR1, or kkα peptides. Splenic T cells from mice immunized with the indicated peptides were cultured with γ-irradiated syngeneic splenocytes pulsed with the indicated peptides (*20*). [³H]thymidine uptake (counts per minute) in triplicate cultures is shown (mean ± SD). One representative result of three different experiments is shown. (**B**) Serum IgG antibodies reactive to cardiac-specific epitopes and ChTR1. Specific antibody production was determined by ELISA (*23*). For each immunization, representative results of three individual mice are shown.

non-CpG ODN was used plus peptide did not induce disease (Table 2). Thus, CpG motif–containing bacterial DNA, including *Chlamydia* DNA, can function as potent immunoactivator for autoimmunity.

Chlamydia pneumoniae has been linked to atherosclerosis and the clogging of blood vessels (*3*, *26*). Experimental *C. pneumoniae* infections in rabbits and mice accelerate atherosclerosis and lead to focal periarteritis (*27*) and *C. trachomatis* infections lead directly to myocarditis (*28*). Mice immunized with *Chlamydia* peptides developed perivascular fibrosis (Fig. 3, A and B), fibrinous occlusions of cardiac blood vessels (Fig. 3, C and D), and thickening of the arterial walls (*29*, *30*). Fibrinous occlusion originating from blood vessel endothelium (Fig. 3C), a minimum of one per individual heart, occurred in 19 out of 32 (60%) hearts analyzed from mice immunized with *Chlamydia*-derived peptides. Similarly, fibrinous occlusion originating from blood vessel endothelium occurred in 14 out of 21 (67%) hearts analyzed from mice immunized with M7Aα. No fibrinous occlusions were detected in hearts from mice immunized with FCA only.

Because activation of autoaggressive T and B cells occurred in the absence of an overt bacterial infection, we then determined whether actual *Chlamydia* infections would lead to the activation of autoaggressive lymphocytes reactive to heart-specific antigens. BALB/c mice were infected with *C. trachomatis* through the respiratory tract and the reproductive organs (*31*). Inflammation of both the respiratory tract or the reproductive organs led to the production of immunoglobulin G (IgG) antibodies to heart-specific epitopes in BALB/c mice (Fig. 4). Because in the mouse model of autoimmune myocarditis, the production of IgG antibodies to heart-specific epitopes is dependent on the activation of autoaggressive T and B cells (*8*), these data show that infection by *C. trachomatis* can activate autoaggressive lymphocytes in BALB/c mice.

Our results lead us to propose that *Chlamydia* infection of an organ can lead to a local immune response followed by systemic activation of autoreactive T and B lymphocytes. Because *Chlamydia* peptides can mimic the effects of heart muscle α myosin heavy chain–derived immunogenic epitopes, T cells activated by *Chlamydia*-derived peptides may trigger organ-specific inflammation within the heart. Dendritic cells, which are resident within the heart and localize in the vicinity of blood vessels, can present cardiac myosin peptides even in healthy animals (*7*). This observation could account for the invasion of autoaggressive T cells that were activated in other organs. In light of the above data, it is conceivable that, during the course of a bacterial infection, the bacterial DNA acts as a potent adjuvant facilitating the activation of autoaggressive T cells (*24*).

In mice, the development of peptide-triggered inflammatory heart disease is related to genetic differences among inbred mouse strains (*6*). Similarly, genetic and environmental risk factors may determine susceptibility to *Chlamydia*-related heart diseases in humans. *Chlamydia* infections are common, and most people can expect to experience a *Chlamydia* infection at least once during their lifetime (*32*). Our data suggest that antigenic mimicry of autoaggressive myosin epitopes by peptides present not only in *C. pneumoniae* but also in *C. trachomatis* and *C. psittaci* may be linked to inflammatory heart disease. Molecular mimicry between bacterial and viral proteins and endogenous molecules has been implicated in various autoimmune diseases, including insulin-dependent diabetes, multiple sclerosis, and autoimmune herpes stromal keratitis (*33*). After initiation of the disease, epitope spreading leads to the maintenance and progression of inflammation. Other mechanisms that could also contribute to the pathogenesis of cardiovascular diseases after *Chlamydia* infection include the production of inflammatory cytokines, bystander activation of lymphocytes, or both (*34*). Our results provide experimental in vivo and in vitro evidence of molecular mimicry between bacterial antigens and heart-specific proteins and indicate that bacterial peptides can trigger tissue-specific inflammation of the heart. In particular, this study establishes a causal link between *Chlamydia* infection and heart disease.

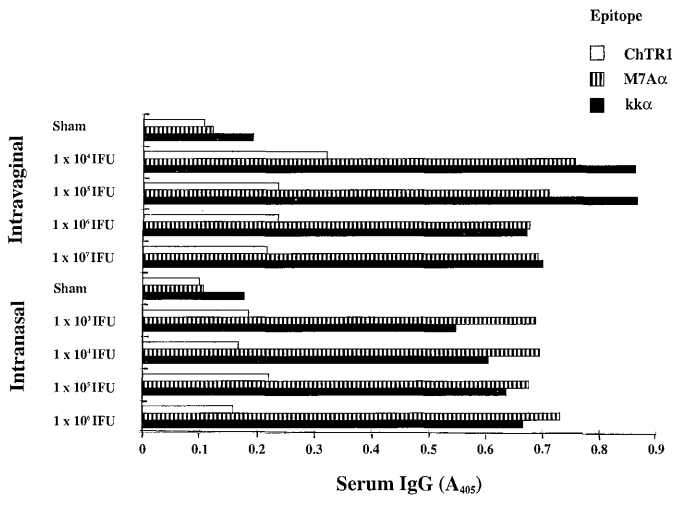


Fig. 4. Serum IgG antibody production in *C. trachomatis*–infected mice. Eight-week-old female BALB/c mice were inoculated either intranasally or intravaginally with the indicated doses of *C. trachomatis* MoPn IFUs (*31*). Thirty-six days (intranasal infection) or 42 days (intravaginal infection) after the inoculation, serum was collected and specific IgG antibody production was determined by ELISA (Fig. 2B). Representative data from individual mice are shown.

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10. The polypeptides were synthesized by FMOC (fluorenyl methoxycarbonyl)–*t*-butyl–based solid-phase peptide chemistry, as described (*8*). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. All peptides were acetylated at the NH_2-terminus. Peptides, dissolved in FCA (1 mg/ml) and emulsified in a 1:1 dilution with phosphate-buffered saline, were injected twice into 6-week-old BALB/c mice (50 μg of peptide per mouse) as described (*8*).
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17. BALB/c mice were immunized twice with ChTR1 in FCA (*10*), and hearts were removed 21 days after the initial immunization. Immunoperoxidase staining of histological heart sections was performed with the following antibodies to rat IgG (all purchased from Pharmingen):

anti-CD11b, anti-CD4, anti-CD8α, and anti-CD45R/B220. Binding of primary antibodies was detected as described (*16*). Percentages of infiltrating cells were calculated from a minimum of 1000 infiltrating cells counted on representative heart sections.
18. Supplemental web material for Fig. 2 (analysis of splenic cell populations) is available at www.sciencemag.org/feature/data/984504.shl.
19. Splenocytes were harvested 21 days after the initial immunization with FCA, ChTR1, or M7Aα, and subpopulations were analyzed by fluorescence-activated cell sorting. Specific antibodies to rat IgG (all purchased from Pharmingen) were as follows: anti-CD11b, anti-CD4, anti-CD8α, and anti-CD45R/B220.
20. Spleens from M7Aα-, ChTR1-, or kkα-immunized BALB/c mice (*10*) were removed 21 days after the first immunization, and T cells were enriched by negatively sorting out CD11b-, Gr1-, and B220-expressing cells with specific antibodies conjugated to magnetic beads (Dynal, Oslo, Norway). In 96-well plates, T cells (1×10^5 T cells per well) were cultured with γ-irradiated syngeneic splenocytes (5×10^5 cells per well) pulsed with M7Aα, ChTR1, or kkα peptide (each at 50 μg/ml). To measure proliferation, we harvested cultures 3 days later after overnight addition of 1 μCi of [^{3}H] thymidine. It should be noted that 10 days after the inital immunization, T cell reactivity is already observed, that is, at a time when no histopathological signs of myocarditis are present.
21. For adoptive transfer of ChTR1 peptide–induced inflammatory heart disease into nonimmunized recipient mice, 6-week-old donor BALB/c mice were immunized twice with ChTR1 peptide in FCA or with FCA only (*10*). Twenty-one days after the initial immunization, splenic T cells (*20*) were cultured with γ-irradiated syngeneic splenocytes pulsed with ChTR1 peptide (50 μg/ml) for 4 days in the presence of mrIL-2 (50 U/ml). Recipient BALB/c mice were injected intraperitoneally with lipopolysaccharide (25 μg per mouse) on days 0 and 4, and 1×10^6 in vitro–stimulated cells from immunized donor mice were injected intravenously on day 7 (*7*). Transferred donor T cells (>95%) had a $TCR\alpha\beta^+CD4^+CD69^+CD25^+$ phenotype.
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23. Twenty-one days after the initial immunization (*10*), serum was collected and specific antibody production was determined by enzyme-linked immunosorbent assay (ELISA) (*6*). Briefly, 96-well plates were coated with 2 μg of peptide per well. Diluted mouse sera (1:100) were allowed to bind to the plates, washed, and binding was detected with horseradish peroxidase–conjugated antibody to mouse IgG (Sigma, #A-3673). Substrate (ABTS, Sigma, #A-1888) conversion was detected measuring absorbance at 405 nm.
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25. CpG motive–containing synthetic oligodeoxynucleotides (ODNs) were derived either from *C. trachomatis* DNA (CpG 1) or from previously reported bacterial DNA sequences (CpGs 2 and 3) (*24*). ODNs were phosphorothioate modified to increase their in vivo stability. ODNs (30 μg in 100 μl of 0.15 mM NaCl buffer) were administered ip at the time of the immunizations. BALB/c mice were subcutaneously immunized twice at a 7-day interval with the M7Aα peptide (50 μg per mouse) in a 1:1 emulsion with mineral oil [Freund's incomplete adjuvant (FIA)] (*8*, *10*). Twenty-one days after the initial immunization hearts were analyzed for the presence and severity of myocarditis.
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29. Supplemental web material for Fig. 3 (arterial wall thickness ratios) is available at www.sciencemag.org/feature/data/984504.shl.
30. For the morphometrical analysis of arteries, inner and outer diameters of individual arteries were measured and the ratio between wall thickness (outer diameter minus inner diameter) and outer diameter was calculated [T. Matsusaka *et al.*, *J. Clin. Invest*, **98**, 1867 (1996)]. At least five arteries with a minimum inner diameter of 50 μm were analyzed per individual heart.
31. The *C. trachomatis* mouse pneumonitis (MoPn) biovar (strain Nigg II) was purchased from the American Type Culture Collection (Rockville, MD) and grown in HeLa-229 cells. Elementary bodies were purified, stored, titered, and prepared for infection as described [S. Pal, I. Theodor, E. M. Peterson, L. M. de la Maza, *Infect. Immun.* **65**, 3361 (1997)]. Eight-week-old female BALB/c mice were inoculated either intranasally with 0 (sham), 1×10^3, 1×10^4, 1×10^5, or 1×10^6 *C. trachomatis* MoPn inclusion–forming units (IFUs), or intravaginally with 0 (sham), 1×10^4, 1×10^5, 1×10^6, or 1×10^7 IFU of *C. trachomatis* MoPn.
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Prion Domain Initiation of Amyloid Formation in Vitro from Native Ure2p

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The [URE3] non-Mendelian genetic element of *Saccharomyces cerevisiae* is an infectious protein (prion) form of Ure2p, a regulator of nitrogen catabolism. Here, synthetic $Ure2p^{1-65}$ were shown to polymerize to form filaments 40 to 45 angstroms in diameter with more than 60 percent β sheet. $Ure2p^{1-65}$ specifically induced full-length native Ure2p to copolymerize under conditions where native Ure2p alone did not polymerize. Like Ure2p in extracts of [URE3] strains, these 180- to 220-angstrom-diameter filaments were protease resistant. The $Ure2p^{1-65}$-Ure2p cofilaments could seed polymerization of native Ure2p to form thicker, less regular filaments. All filaments stained with Congo Red to produce the green birefringence typical of amyloid. This self-propagating amyloid formation can explain the properties of [URE3].

Genetic evidence identified [URE3] and [PSI], two nonchromosomal genes of *Saccharomyces cerevisiae*, as prions of Ure2p and Sup35p, respectively, which implies that proteins can be hereditary material (*1*). In response to a good nitrogen source (ammonia or glutamine), Ure2p blocks assimilation of poor nitrogen sources by blocking the action of the transcription regulator Gln3p (*2*). Sup35p is a subunit of the translation release factor (*3*). [URE3] (*4*) and [PSI] (*5*) are altered forms of Ure2p and Sup35p that have lost their normal functions but have acquired the ability to convert their normal forms into the altered (prion) form (*1*), a notion supported by genetic and biochemical data (*6–10*). The prion concept originates in studies of the spongiform encephalopathies (*11*), believed due to a self-propagating altered form of PrP that forms scrapie-associated filaments and amyloid deposits in brains of affected animals (*12*).

Amyloid is defined as a filamentous protein structure that stains with the dye Congo Red (CR) to produce green birefringence under polarized light and is characterized by protease resistance and an antiparallel β sheet structure (*13*). Amyloid deposits of the Aβ peptide ac-

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