EFFECT OF BLOCKING THE CXCL9/10-CXCR3 CHEMOKINE SYSTEM IN THE OUTCOME OF ENDOTHELIAL-TARGET RICKETTSIAL INFECTIONS

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Abstract. Rickettsiae cause systemic infections such as Rocky Mountain spotted fever and boutonneuse fever. The main cellular target of these obligately intracellular bacteria is the endothelium. T lymphocytes are the most important effectors of immunity, and the CXCR3 ligands CXCL9 and CXCL10 may play an important role in the T cell-mediated clearance of rickettsiae from the infected vasculature as suggested by recent expression studies. Here we showed that antibody-mediated neutralization of CXCL9 and CXCL10, and CXCR3 gene knockout, had no effect on survival or bacterial loads of mice infected with rickettsiae. We also demonstrated that rickettsiae triggered the endothelial expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 *in vivo*. These findings suggested that antigenic presentation by endothelial cells together with an endothelial inflammatory phenotype induced by the rickettsial infection may be sufficient to arrest T cells and trigger their anti-rickettsial effector mechanisms without the need for chemokines.

INTRODUCTION

Rickettsiae of the spotted fever group are gram-negative, obligately intracellular bacteria, transmitted mainly by ticks. Their primary cellular targets are endothelial cells, and they spread from cell to cell through stimulation of directional actin polymerization.¹⁻⁴ They cause acute, potentially lethal diseases with systemic multi-organ involvement such as Rocky Mountain spotted fever (RMSF caused by Rickettsia rickettsii), and boutonneuse fever (caused by R. conorii).⁵ If not treated early with appropriate antibiotics, these diseases (particularly RMSF) can be fatal. The initial symptoms are those of a flu-like syndrome. With delayed treatment or no treatment, severe manifestations can develop including delirium, seizures, coma, noncardiogenic pulmonary edema/ adult respiratory distress syndrome, interstitial pneumonia, acute renal failure, hemorrhagic phenomena, peripheral edema, and hypovolemic hypotension due to leakage of intravascular fluid into the extravascular space.⁶

In animal models CD8+ T lymphocytes and their effector mechanisms, interferon- γ (IFN- γ) production and cytotoxicity, are required for an effective anti-rickettsial immune response.^{7,8} In humans, similar mechanisms are likely to operate as suggested by the presence of perivascular infiltration of CD8+ T cells in skin lesions of patients with spotted fever group rickettsioses.9 One important aspect of the anti-rickettsial immune response that remains unknown is the mechanism(s) whereby T cells localize and focalize their effector functions to infected endothelial cells. We hypothesize that chemokines play a crucial role, particularly inflammatory chemokines that specifically target activated T cells. In this regard, we recently found that the CXCR3 ligands, CXCL9 (Mig) and CXCL10 (IP-10), are expressed at much higher levels than other T cell-targeting chemokines in a mouse model of spotted fever group rickettsioses.¹⁰ In the present work, we investigated the possible functional role of CXCL9 and CXCL10 in the immune response against spotted fever group rickettsiae.

MATERIALS AND METHODS

Rickettsiae. *Rickettsia conorii* (Malish 7 strain) is a human isolate from South Africa with an unknown number of passages in the yolk sacs of embryonated chicken eggs. For all the

experiments described in this study, a stock of R. conorii was produced by cultivation in specific pathogen-free embryonated chicken eggs. Yolk sacs from infected eggs with dead embryos were homogenized in a Waring blender, diluted to a 10% suspension in sucrose-phosphate-glutamate buffer (SPG; 0.218 M sucrose, 3.8 mM KH₂PO4, 7.2 mM K₂HPO₄, 4.9 mM monosodium L-glutamic acid, pH 7.0) and aliquoted for storage at -80°C after discarding the pellet produced by low speed centrifugation ($200 \times g$ for 10 minutes). Rickettsial content of this stock was quantified by plaque assay (5.5×10^7) plaque-forming units [pfu]/mL),¹¹ and the 50% lethal dose (LD₅₀) was determined experimentally in C3H/HeN mice $(1 \text{ LD}_{50} = 1.65 \times 10^4 \text{ pfu/mL})$ according to the method of Reed and Muench.¹² Rickettsia australis (Cutlack strain) was a gift from C. Pretzman (Department of Health Laboratory, Columbus, OH) and was passaged three times in Vero cells (ATCC CCL81; American Type Culture Collection, Manassas, VA) and five times in embryonated chicken yolk sacs in our laboratory. The LD₅₀ of the egg yolk sac suspension of *R. australis* in 10% SPG buffer $(2 \times 10^6 \text{ pfu/mL})$ was determined experimentally in C57BL/6 mice (1 LD₅₀ = 1×10^4 pfu/ml).

Animal models. We used two mouse models of endothelialtarget spotted fever group rickettsioses. One model, R. conorii infection of C3H/HeN mice, has been well characterized.13 The other is *R. australis* infection of C57BL/6 mice.^{8,14} All mice were housed in a biosafety level 3 facility and were inoculated intravenously (through the tail vein) with different doses as indicated in the results. For antibody neutralization experiments, mice received 0.6 mg of purified rabbit IgG (0.3 mg each of anti-CXCL9 and anti-CXCL10, or 0.6 mg of control IgG) intraperitoneally starting 24 hours before inoculation with rickettsiae and then subsequently every other day for a total of four doses. The CXCR3 gene knockout mice were generously provided by Bao Lu and Craig Gerard (Perlmutter Laboratory, Children's Hospital and Harvard Medical School, Boston, MA). They were bred according to protocols of the Animal Resource Center of the University of Texas Medical Branch.

Antibodies. Rabbit polyclonal antibodies to CXCL9 and CXCL10 were produced by Biosynthesis (Lewisville, TX) using synthetic peptides selected from the respective protein sequences coupled to carrier keyhole limpet hemocyanin

(KLH) as described previously.^{15–17} Antibodies against these peptides neutralize CXCL9 and CXCL10.^{15–17} The IgG fraction was purified with protein A columns (Pierce Biotechnology, Rockford, IL).

Endothelial cell line. The C3H/HeN mouse endothelial cell line SVEC4–10 was kindly provided by Dr. M. Edidin (Johns Hopkins University, Baltimore, MD).¹⁸ This cell line was cultivated in Dulbecco's modified minimal essential medium (DMEM) (Gibco Life Technologies, Carlsbad, CA) containing 2% fetal bovine serum (Hyclone Inc, Logan, UT). The cells were passaged twice a week.

T cell lines. A single cell suspension was produced from aseptically extracted spleens of R. conorii-immunized mice; 2×10^7 splenocytes were seeded in each well of a six-well plate containing formalin-fixed R. conorii-infected, histocompatible endothelial cells (SVEC4-10 cells). A monolayer of the endothelial cells had been infected with R. conorii two days earlier and fixed with 2% formalin in phosphate-buffered saline for 20 minutes at 4°C. The excess formalin was blocked with lysine solution (1 part 0.4 M L-lysine, pH 7.4, and one part DMEM with 10% bovine calf serum) for 30 minutes and subsequently washed four times with DMEM containing 10% bovine calf serum (Gibco Life Technologies) before adding the immune splenocytes. One week later, dead cells were separated from live cells by density gradient centrifugation in NycoPrep 1.077A (Axis-Shield, Oslo, Norway). Live immune cells were then mixed with irradiated splenocytes (2,300 rad) from naive mice in a proportion of 1:5 and added to formalin-fixed, infected SVEC4-10 cells in the presence of 10 units/mL of recombinant human interleukin-2 (IL-2) (Roche, Indianapolis, IN). The T cells were re-stimulated every week in the same manner.

Quantitative real-time polymerase chain reaction (PCR). This was performed using the iCycler (BioRad, Hercules, CA). The rickettsial load was determined by a real-time PCR (with Taqman[®] probes [Biosearch Technologies, Novato, CA] for R. conorii and SYBRgreen® [Stratagene, La Jolla, CA] for R. australis) of the Rickettsia-specific outer membrane protein B (rompB) gene. For these experiments, the substrate of amplification was DNA purified from frozen samples using the Dneasy Tissue kit from Qiagen (Valencia, CA). The results were normalized to glyceraldehyde-3phosphate dehydrogenase (GAPDH) in the same sample and expressed as copy number per 10⁵ copies of GAPDH (standard curves with more than 94% efficiency and linear amplification from 1 to 10^{6} – 10^{7} copies were used to obtain the copy number of the samples). The sequences of the primers and probes are R. conorii rompB forward, 5'-ACACATGCTGC-CGAGTTACG-3'; R. conorii rompB reverse, 5'-AATTGT-AGCACTACCGTCTAAGGT-3'; R. conorii rompB probe, 5'-CGGCTGCAAGAGCACCGCCAACAA-3'; R. australis rompB forward, 5'-GCCGAGTTACGTTTAGGAAATG-3'; R. australis rompB reverse, 5'-CGGTAATTGTAGCACTT-CCATCT-3'; GAPDH forward, 5'-CAACTACATGGTC-TACATGTTC-3'; GAPDH reverse, 5'-CTCGCTCCTGGA-AGATG-3'; and GAPDH probe, 5'-CGGCACAGTCAAG-GCCGAGAATGGGAAGC-3'. The R. conorii rompB probe was labeled with FAM and Black Hole Quencher 1TM (BHQ1), and the probe for GAPDH was labeled with TAMRA and Black Hole Quencher 2TM (BHQ2) (Biosearch Technologies).

Immunohistochemistry. Sections from zinc-fixative containing tissues (4–6 μ m) were examined for the presence of CD3+ T cells with a rat anti-mouse CD3 monoclonal antibody (Clone CD3–12; Serotec, Raleigh, NC) as primary antibody, biotinylated F(ab')₂ fragments of mouse-absorbed, mouse anti-rat IgG (Jackson Immunoresearch, West Grove, PA) as a secondary reagent, avidin-biotin complex (ABC) peroxidase as the third reagent, and 3,3'-diaminobenzidine (DAB) as a substrate (Vector Laboratories, Burlingame, CA).

RESULTS

Survival and rickettsial load of naive mice receiving neutralizing anti-CXCL9/10 antibodies. We administered neutralizing antibodies against CXCL9 and CXCL10 to mice infected with a sublethal inoculum (0.25 LD_{50}) of *R. conorii* to determine if this chemokine system played a role in the clearance of rickettsiae from the vascular endothelium. The antibodies were made against peptides that had been shown to stimulate the production of neutralizing antibodies,^{15–17} and the dose of R. conorii was determined in a pilot experiment in which infectious doses lower than expected caused death of mice receiving rabbit IgG. Rabbit IgG by itself did not produce any effect. In two separate experiments, there was no difference in survival between R. conorii-infected mice receiving anti-CXCL9/10 and control animals receiving purified rabbit IgG (Table 1). Furthermore, when rickettsial loads were determined in lung and liver by a quantitative real-time PCR, we found that there were no statistically significant differences between mice receiving the chemokine-neutralizing antibodies and those receiving control rabbit IgG (Figure 1).

Survival and rickettsial load of naive mice receiving neutralizing anti-CXCL9/10 antibodies and adoptively transferred anti-R. conorii T cells. Since it is possible that the consequences of neutralizing CXCL9 and CXCL10 is not apparent in mice receiving a sublethal inoculum of R. conorii, we administered the anti-CXCL9/10 antibodies to mice receiving 3 LD_{50} of *R. conorii* 24 hours after the adoptive transfer of 10^7 anti-R. conorii T cells from a T cell line. In addition, this experimental design allowed us to test the possible role of these chemokines during the effector phase of the anti-rickettsial immune response. All mice receiving immune T cells survived whether they received anti-CXCL9/10 antibodies, control rabbit IgG, or no antibody treatment, while control mice receiving 3 LD₅₀ of *R. conorii* and no other treatment succumbed to the infection by day 6 (Table 2). Although the rickettsial loads on day 6 in lungs and livers were lower in animals receiving neutralizing anti-CXCL9/10 antibodies, the fact that the animals not killed (for determination of bacterial loads) survived, indicated that these differences were not biologically critically important (Figure 2).

TABLE 1 Effect of neutralization of CXCL9/10 on survival of mice receiving 0.25 LD₅₀ of *Rickettsia conorii**

Antibody	Survival
No antibody	10/10
Control rabbit IgG	8/10
Rabbit IgG anti-CXCL9/10	8/10

* $LD_{50} = 50\%$ lethal dose.



FIGURE 1. Quantitative real-time polymerase chain reaction with Taqman[®] probes of DNA from livers (**A**) and lungs (**B**) of naive C3H/HeN mice infected intravenously with 0.25 50% lethal doses of *Rickettsia conorii*. The number of copies of the rickettsial outer membrane B (*rompB*) gene was normalized by expressing this value to 10^5 copies of the eukaryotic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. All data points correspond to the mean \pm SD of five different animals, and for each mouse we performed three simultaneous amplifications with negligible standard deviations. The peak of rickettsial load usually occurred on day 5, and this explains the apparent lack of difference between days 4 and 6.

Survival and rickettsial load in CXCR3 gene knockout mice. To further evaluate the roles of CXCL9/10 on the induction of the anti-rickettsial T cell response and/or the effector phase of the adaptive immune response, we challenged mice that did not possess the receptor for CXCL9 and CXCL10 (CXCR3) with 0.5, 1, or 3 LD₅₀ of *R. australis*. We used *R. australis* because the C57BL/6 strain of mice (the

TABLE 2

Effect of anti-CXCL9 and anti-CXCL10 on survival of mice receiving *Rickettsia conorii*-immune T cells and 3 LD₅₀ of *R. conorii**

Antibody	Survival
No antibody	8/8
Control rabbit IgG	8/8
Rabbit IgG anti-CXCL9/10	8/8
No T cells	0/5

* $LD_{50} = 50\%$ lethal dose.



FIGURE 2. Quantitative real-time polymerase chain reaction with Taqman[®] probes of DNA from livers (**A**) and lungs (**B**) of naive C3H/HeN mice receiving anti-*Rickettsia conorii* T cells and intravenously infected with 3 50% lethal doses of *R. conorii*. Antibody treatments are indicated in the graphs. The number of copies of the rickettsial outer membrane B (*rompB*) gene was normalized by expressing this value to 10^5 copies of the eukaryotic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. All data points correspond to the mean \pm SD of three different animals, and for each mouse we performed three simultaneous amplifications with negligible standard deviations. The peak of rickettsial load usually occurred on day 5, and this explains the apparent lack of difference between days 4 and 6.

genetic background of the CXCR3 gene knockout mice) is susceptible to this species of *Rickettsia*, while C3H/HeN mice, the strain used for the other experiments, are susceptible to *R. conorii*. We found no difference in survival between CXCR3 gene knockout mice and wild type mice at the three different doses of *R. australis* examined (Table 3). In addition, although there was a higher rickettsial load in the lungs of CXCR3 KO mice on day 6 of infection when compared with wild type animals, by day 8 this difference was no longer apparent, and rickettsiae had been almost completely cleared. In the liver, six days after *R. australis* infection, wild type animals had higher rickettsial loads in one of two separate experiments; however, similar to the lungs, by day 8 there were no significant differences in rickettsial load, and rickettsiae had been almost completely eliminated (Figure 3).

Rickettsia australis	Survival	
	Wild type	CXCR3 KC
0.5 LD ₅₀	5/5	5/5
$1 LD_{50}$	3/8	4/8
$3 LD_{50}^{50}$	0/5	0/5

* $LD_{50} = 50\%$ lethal dose.

T Cell infiltration in CXCR3 gene knockout mice and mice receiving neutralizing anti-CXCL9/10 antibodies. When we analyzed the pattern of infiltration of CD3+ T cells in infected tissues of C3H/HeN mice receiving neutralizing anti-CXCL9/10 antibodies or in infected tissues of CXCR3 gene knockout mice, we found no differences when compared to control animals (Figure 4). This indicates that anti-rickettsial T cells cir-



FIGURE 3. Quantitative real-time polymerase chain reaction with SYBRgreen[®] of DNA from livers (**A**) and lungs (**B**) of wild type (WT) and CXCR3 knockout (KO) mice intravenously infected with 0.5 50% lethal doses of *R. australis*. The number of copies of the rickettsial outer membrane B (*rompB*) gene was normalized by expressing this value to 10^5 copies of the eukaryotic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. All data points correspond to the mean \pm SD of five different animals, and for each mouse we performed three simultaneous amplifications with negligible standard deviations.

culate and infiltrate tissues normally despite the inhibition or absence of this chemokine system.

Expression of the adhesion molecules intercellular adhesion molecule 1(ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). The surprising minimal effect of neutralizing the T cell targeting chemokines CXCL9/10 or of genetic absence of their receptor CXCR3 in the clearance of rickettsiae from the infected endothelium led us to hypothesize that when the endothelium is the target of infection (as it is the case for most rickettsial infections) and presents antigen to T cells, this chemokine system is not essential because inflammatory adhesion molecules in conjunction with antigen presentation are sufficient to trigger the effector functions of immune T cells. Indeed, we found that the adhesion molecules ICAM-1 and VCAM-1 were both expressed by the endothelium of all organs examined starting on day 2 of a rickettsial infection as assessed by immunohistochemical analysis with specific antibodies (Figure 5). This increased expression declined on day 15; however, at the last day of examination (day 20) it had not returned completely to basal levels as assessed by immunhistochemical analysis. VCAM-1 and ICAM-1, which are normally expressed under non-inflammatory conditions in the endothelium of the lung, were not expressed in the other organs of the uninfected mice.

DISCUSSION

We had recently reported that the CXCR3 ligands CXCL9 and CXCL10 were expressed at much higher levels than other T cell-targeting chemokines during the course of infections caused by the spotted fever group rickettsia R. conorii.¹⁰ These chemokines target effector/memory T cells more specifically,¹⁹ and T cells, particularly CD8+ T cells, have been shown to be vital in the clearance of rickettsiae from infected endothelium (the main target cells).⁸ There is also suggestive evidence from other systems, including cell culture studies involving flow of the medium,²⁰ transplantation studies,^{21–23} and other infectious diseases caused by intracellular organisms that do not target endothelial cells,^{14,15,24} that chemokines might play an important role in the anti-rickettsial immune response. For instance, antibody-mediated neutralization of CXCL10 in a mouse model of toxoplasmosis results in increased parasite burden and mortality.²⁴ In fact, anti-CXCL10 treatment reduced the number of antigen-specific splenic cytotoxic lymphocytes. Also, CXCL9 and CXCL10 neutralization drastically reduced the number of protective CD4+ and CD8+ T cells infiltrating into the central nervous system of mice with acute encephalomyelitis caused by mouse hepatitis virus.^{15,16} These data, and our recent results regarding CXCL9 and CXCL10, led us to investigate the effect of antibody-mediated chemokine neutralization and knockout of the gene for their receptor (CXCR3) in survival and clearance of rickettsiae using mouse models that faithfully mimic human rickettsioses.8, 13

We first showed that neutralization of CXCL9 and CXCL10 in C3H/HeN mice receiving an ordinarily sublethal inoculum of *R. conorii* (0.25 LD₅₀) did not result in higher mortality (Table 1) or reduced rickettsial clearance (Figure 1) when compared to control animals. In a pilot experiment, we had determined that 0.25 LD₅₀ (for untreated mice) was the highest dose that we could give to animals receiving rabbit



FIGURE 4. Immunohistochemical analysis for the T cell marker Cd3 (**brown**). **A**, Liver of a C3H/HeN mouse that received neutralizing antibody IgG anti-CXCL9/10 and 0.25 50% lethal doses (LD_{50}) of *Rickettsia conorii* (day 6 after infection). Control animals showed a similar pattern of infiltration. **B**, Lung of a CXCR3 gene knockout mouse infected five days earlier with 1 LD_{50} of *R. australis*. Wild type C57/BL6 mice showed a similar pattern of T cell infiltration. (Magnification × 200.).

IgG without causing substantial mortality, indicating that the presence of this xenogenic protein modified the course of the experimental rickettsial infection. To bypass the immune priming process and investigate the effect of neutralizing CXCL9 and CXCL10 during the effector phase of the immune response in the presence of a lethal inoculum of *R. conorii*, we adoptively transferred anti-*R. conorii* T cells from a T cell line 24 hours before inoculating with 3 LD₅₀ of *R. conorii*. All mice receiving immune T cells survived irrespective of the treatment, while control animals that did not receive T cells died around day 6 as expected (Table 2). In addition, there were no biologically important differences in the rickettsial loads of two important target organs, lungs and liver (Figure 2).

The effect of CXCL9 and CXCL10 on the outcome of infections with *R. conorii* was also analyzed by the survival and rickettsial loads of mice that do not express the receptor for these chemokines (CXCR3 gene knockout mice) after infection with *R. australis* (the only spotted fever group rickettsial species that causes disease in the C57BL/6 strain of mice).

Indeed, we observed no significant differences between wild type animals and CXCR3 gene knockout mice, both in terms of survival (Table 3) and rickettsial loads (Figure 3). The higher rickettsial loads found in the lungs of CXCR3 gene knockout mice on day 6 suggested that CXCL9, CXCL10, and CXCL11 may play some role in host defense. Also, the differences in rickettsial loads in the liver were opposite to those found in the lungs, suggesting that the CXCR3 ligands may play a role in the local immune response of the liver, although opposite to that of the lungs. In any case, the function of CXCL9, CXCL10, and CXCL11 is probably redundant with other chemokine or inflammatory systems since rickettsial loads on day 8 are very low in the lungs and livers of both wild type animals and CXCR3 gene knockout mice. Finally, the lack of effect of the CXCL9/10/11-CXCR3 system in the trafficking of anti-rickettsial T cells was evident in the normal infiltration of CD3+ T cells in infected tissues of both CXCR3 gene knockout mice and C3H/HeN mice receiving neutralizing anti-CXCL9/10 antibodies (Figure 4).

Chemokines trigger firm adhesion to the endothelium and



FIGURE 5. Immunohistochemical analysis (**brown**) for intercellular adhesion molecule 1 (**A**) and vascular cell adhesion molecule 1 (**B**) in the brain of a C3H/HeN mouse infected with 0.25 50% lethal doses of *Rickettsia conorii* four days earlier. Uninfected animals showed no detectable signal. (Magnification \times 200.)

guide the migration of leukocytes^{25,26} in infections that almost always involve cells other than endothelial cells. Rickettsiae are among the few microorganisms that infect mainly endothelial cells. As a consequence of rickettsial infection, these cells express an inflammatory phenotype which includes cytokines such as IL-1 α , IL-6, and IL-8, 27,28 and adhesion molecules including ICAM-1, VCAM-1,²⁹ and endothelialleukocyte adhesion molecule 1.30 Our results led us to hypothesize that antigenic presentation by endothelial cells in the context of increased expression of inflammatory adhesion molecules might be sufficient to trigger the effector mechanisms of anti-rickettsial T cells and in this way may bypass the requirement for chemokines. In support of this hypothesis, the present study demonstrated that the expression of ICAM-1 and VCAM-1, previously reported using in vitro models, as a consequence of a rickettsial infection,²⁹also occurs in vivo in an animal model of spotted fever group rickettsiosis (Figure 5). Furthermore, it has been shown that ICAM-1 at higher densities increases lymphocyte adhesion without the need for chemokine agonists, although adhesion is further increased in the presence of chemokines through triggering of a high affinity state for the ICAM-1 ligand leukocyte function-associated antigen 1(LFA-1) (CD11aCD18).³¹ This higher affinity state of LFA-1 can also be stimulated through antigen recognition with the T cell receptor (TcR),³² and with triggering of CD2 or CD3.33 Similarly, stimulation through the TcR causes increased adhesion of lymphocytes through the VCAM-1 ligand very late antigen 4 (VLA-4).^{34,35} Therefore, when T lymphocytes recognize rickettsial antigen presented by infected endothelial cells, the combination of increased expression of endothelial integrins (such as ICAM-1 and VCAM-1) and increased affinity of ligands such as LFA-1, as a consequence of TcR and CD3 signaling, should be sufficient to arrest and activate T cells, thus bypassing the need for chemokines.

Recently, it was demonstrated in vivo that endothelial cells drive the homing of activated T cells though presentation of the antigen for which those cells are specific, together with the expression of the non-inflammatory chemokine CCL21 (SLC).³⁶ Both elements are necessary since absence of major histocompatility class (MHC) class I or antibody-mediated neutralization of CCL21 prevented the homing of the lymphocytes. These data are particularly relevant to rickettsioses because they show that antigenic presentation through MHC class I should be one element of the specific homing of rickettsia-specific T cells. In addition, these experiments show that a chemokine that was thought to be involved only in the trafficking of lymphocytes to secondary lymphoid organs is expressed by non-lymphoid endothelium under non-inflammatory conditions. The expression of CCL21 by endothelial cells in non-lymphoid organs has been confirmed in humans with T cell-infiltrative skin diseases,³⁷ the autoimmune diseases rheumatoid arthritis and ulcerative colitis,38 and in the blood brain barrier of mice with experimental allergic encephalomyelitis.³⁹ Although Savinov and others³⁶ demonstrated that CCL21 is required for the homing of antigenspecific CD8+ T cells under non-inflammatory conditions, in the rickettsial model, even this chemokine might not be essential due to the inflammatory phenotype of infected endothelial cells. This hypothesis needs to be addressed with functional studies of rickettsia-infected endothelial cells under conditions of flow. As part of such studies, it will be important to determine if antigen presentation by endothelial cells occurs on the luminal side, the same side where integrins are expressed.

In conclusion, we have demonstrated that the chemokine system CXCL9/10-CXCR3, which has the highest level of expression among other T cell-targeting chemokines during the course of experimental rickettsial infections,¹⁰ is not required to clear rickettsiae from the vascular endothelium. This result is surprising since this system has been shown to be essential for the immune clearance of other pathogens causing intracellular infections. We propose that rickettsioses present a unique scenario because of the type of target cell and rickettsia-triggered expression of endothelial adhesion molecules such as ICAM-1 and VCAM-1, which together with antigenic presentation by molecules of the MHC expressed by the endothelium could provide the signals necessary to activate the effector mechanisms of T cells.

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