Lymphadenopathy in a Novel Mouse Model of *Bartonella*-Induced Cat Scratch Disease Results from Lymphocyte Immigration and Proliferation and Is Regulated by Interferon- α/β

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In immunocompetent humans, cat scratch disease (CSD) is elicited by the Gram-negative bacterium *Bartonella henselae* and is characterized by a benign regional lymphadenopathy, the pathogenesis of which is poorly understood. Here, we describe a novel mouse model of *Bartonella*-induced CSD-like disease that allowed us to investigate the mechanisms leading to lymphadenopathy *in vivo*. In wild-type mice, a subcutaneous inoculation of either viable or inactivated *B. henselae* led to a strong swelling of the draining lymph node, which was long-lasting despite the rapid elimination of the bacteria. Carboxyfluorescein- and bromodesoxyuridine-labeling experiments showed that lymph node enlargement resulted from modified immigration and enhanced proliferation of lymphocytes, preferentially of B cells. A comparative analysis of *B. henselae* and the rodent pathogen *B. grabamii* in wild-type versus interferon- α/β -receptor I chain-deficient mice revealed that interferon- α/β is not only differentially induced by these two *Bartonella* species but also exerts an inhibitory effect on the development of lymphadenopathy both *in vitro* and *in vivo*. These data demonstrate that the lymphadenopathy of human CSD can be reproduced and studied in a mouse model and provide the first insights into the underlying immunological mechanisms. (Am J Pathol 2008, 172:1005–1018; DOI: 10.2353/ajpath.2008.070591)

Cat scratch disease (CSD) of humans is a common zoonosis throughout the world, which is characterized by a

benign and self-limiting regional lymphadenopathy.¹ It is mainly elicited by *Bartonella henselae*, a small and fastidious Gram-negative rod belonging to the group of α -proteobacteria. The domestic cat and other felids serve as reservoir host for this facultative intracellular bacterium.^{2,3} The cat flea (*Ctenocephalides felis*) is considered as the most important vector transmitting the bacterium from its chronic bacteremic reservoir host to accidentally infected humans.⁴

In human hosts *B. henselae* can induce different clinical manifestations depending on the individual immune status. Viable *B. henselae* are usually detected only in immunocompromised patients, in whom the bacteria spread and cause vasoproliferative disorders such as bacillary angiomatosis and peliosis.^{5,6} In immunocompetent humans, in contrast, the inoculation of *B. henselae* either leads to an abortive infection or causes CSD.^{7–9} Most CSD patients develop a primary lesion at the site of infection followed by the characteristic regional lymphadenopathy that persists for weeks to months and may suppurate.^{10,11} In a minority (5 to 20%) of *B. henselae*-infected immunocompetent patients atypical manifestations of CSD (with or without lymphadenopathy) such as systemic infection with fever, hepatosplenomegaly, encephalitis, or osteomyelitis can be seen.^{10,11} Infected individuals produce high amounts of *B. henselae*-specific antibodies¹² and specific granulomata may form in affected tissues.^{10,13–15} Because CSD is a self-limiting disease, its clinical relevance mainly results from the necessity to exclude other infectious or malignant processes.

Little is known about the pathogenesis of the long-lasting lymphadenopathy induced by *B. henselae*. Be-

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cause viable bacteria have only rarely been isolated from affected lymph nodes (LNs) of CSD patients,⁹ an immunopathogenesis is assumed.¹⁶ A recently published study addressed the role of human dendritic cells (DCs) in CSD and proposed that *B. henselae*-infected DCs locally produce cytokines [(interleukin (IL)-10] and chemokines (CXCL13) that contribute to the formation of the characteristic B cell and neutrophil-rich CSD granulomata.¹⁷ Considering the limitations of human studies, a few groups attempted to set up CSD mouse models. Similar to the situation in the human host *B. henselae* was rapidly eliminated from mice after systemic (intraperitoneal or intravenous) inoculation. An involvement of LNs was not reported in these studies.^{18–22} Thus, a valid animal model for *B. henselae*-induced lymphadenopathy that would allow further investigation of the pathogenesis of CSD does not exist to date.

In the present report we describe a novel mouse model that mimics human CSD. In contrast to all previous studies the mice were infected locally (ie, subcutaneously) with *B. henselae* or *B. grahamii*, a rodent-specific *Bartonella* species. In both cases a regional lymphadenopathy developed that—especially in the case of *B. henselae* infection—resembled human CSD with respect to duration and severity. We analyzed the cellular composition and morphology of the affected LNs and investigated the trafficking, proliferation, and cytokine production of immune cells to provide insights into the mechanism(s) underlying the LN swelling.

Materials and Methods

Mice, Bacteria, and Infection

Female C57BL/6 or BALB/c mice (Charles River Laboratories, Sulzfeld, Germany), mice deficient for the IFN- α / β -receptor chain I (IFNARI^{-/-}) (10th generation backcross to C57BL/6, kindly provided by Ulrich Kalinke, Paul-Ehrlich-Institut, Langen, Germany) and mice deficient for the recombinase activating gene 1 (RAG1^{-/-}) (22nd generation backcross to C57BL/6; The Jackson Laboratory, Bar Harbor, ME) were kept under specific pathogen-free conditions. Mice were used at the age of 6 to 12 weeks and experiments were conducted according to the German animal protection law.

Three different strains of *B. henselae* (Fr98/K8, isolated from the blood of a cat of a CSD patient; Fr98/K8aM, isolated from the spleen of a *B. henselae* Fr98/K8-infected C57BL/6 mouse 3 weeks after infection; ATCC 49882) and *B. quintana* (strain Toulouse) were grown on chocolate agar plates containing 5% defibrinated sheep blood and 0.9 μ mol/L hemin. *B. grahamii* (strain ATCC 700132) was cultured on agar plates containing 10% defibrinated sheep blood at 37°C and 5% CO₂. The growth of *B. henselae* Fr98/K8 and Fr98/K8aM was supported by culturing *Staphylococcus epidermidis* on the edge of the agar plates. Bacteria were harvested from the agar plates by gentle scraping after 4 (*B. henselae* and *B. quintana*) or 3 days (*B. grahamii*) of culture for *in vivo* infection experiments and 1 day earlier for *in vitro* studies. Before use

they were washed and resuspended in sterile, endotoxin-free phosphate-buffered saline (PBS). The number of colony-forming units (CFU) in bacterial suspensions was estimated by comparing their optical density at 600 nm to a standard curve. The exact CFU of the suspensions was determined by plating 10-fold serial dilutions. For some experiments bacteria were inactivated by either heat (95°C, 15 minutes) or sonication (3 $\times$ 5 minutes on ice, pulsed, energy output level 6; Sonifier Cell Disrupter B15; Branson, Danbury, CT). The inactivation was controlled by plating an aliquot of treated suspensions.

For infection, different doses of bacteria (1 to 4 $\times$ 10⁸ CFU in a volume of 20 μ l) were inoculated subcutaneously in the dorsal side of the hind foot of the mice. The following inocula were used as controls: 1) PBS alone; 2) sham controls using *Bartonella*-free agar plates (with or without *S. epidermidis*) that were processed exactly as those plates from which *Bartonella* was harvested by gentle scraping; 3) material scraped off the upper layer of agar plates without *B. henselae* and resuspended in PBS; and 4) *B. henselae* Fr98/K8aM harvested from plates without *S. epidermidis*. One day to fifteen weeks after challenge mice were sacrificed and whole blood, serum, and the spleen, liver, and draining popliteal LN (popLN) were obtained from each mouse for analysis.

Culture and Polymerase Chain Reaction (PCR) Detection of *Bartonella* from Infected Mice

From each mouse the draining popLNs as well as weighed parts of spleen and liver were homogenized in PBS and plated along with at least 200 μ l of blood. Cultures were incubated for 3 weeks at 37°C and 5% CO₂. Colonies with typical *Bartonella*-like morphology were counted and the number of CFU per gram of tissue or μ l of blood was calculated.

Genomic DNA of popLNs, spleen, liver, and blood was extracted using the NucleoSpin tissue kit (Macherey-Nagel, Düren, Germany). *Bartonella*-specific DNA was detected in a real-time PCR (Light Cycler; Roche Diagnostics, Mannheim, Germany) amplifying the RiboflavinC gene. Amplification was performed in 20 μ l of Quantitect Probe PCR master mix (Qiagen, Hilden, Germany) containing 0.5 μ mol/L of the primers LC-RibC-F (5'-GGTGCATCAATTGCGTGTTC-3') and LCN-RibC-R (5'-ATGCCACCC-ATTCATCACC-3') as well as 0.2 μ mol/L of the labeled probe 6-carboxyfluorescein (FAM)-5'-TTGGTTTGCTGTGAAGCGTGGGAAGA-3'-6-carboxytetra-methylrhodamine (TAMRA) (all purchased from MWG Biotech, Ebersberg, Germany). Thermal cycling was performed with an initial denaturation step at 95°C for 15 minutes, followed by 95°C for 15 seconds, and 58°C for 1 minute for 45 cycles. Data were analyzed using the LightCycler software (version 3.5).

Detection of *B. henselae*-Specific Antibodies

The analysis of mouse sera for the presence of *B. henselae*-specific antibodies was performed microscopically with a commercially available immunofluorescence test (Bios

GmbH, Munich, Germany). A goat anti-mouse IgG-fluorescein isothiocyanate-labeled antibody (Jackson ImmunoResearch Europe Ltd., Soham, UK) was used for detection.

Histology

For histopathological analyses cryosections (5 μ m) of popLNs were stained with hematoxylin and eosin (H&E). Immunohistochemical stainings of acetone-fixed cryosections (5 μ m) were performed using unconjugated rat anti-CD45R/B220 (RA3-6B2), rat anti-CD11b (M1/70), rat anti-Ly6G/C (Gr-1, RB6-8C5) (all BD Biosciences, Heidelberg, Germany), rat anti-CD4 (YTS 191.1.2, hybridoma supernatant²³), rat anti-CD8 α (YTS 169.4, hybridoma supernatant²³), rat anti-F4/80 (C1:A3-1; BMA Biomedicals, Augst, Switzerland) and hamster anti-CD11c (N418; AbD Serotec, Düsseldorf, Germany) monoclonal antibodies (mAbs). Primary mAbs were followed by biotinylated mouse anti-rat IgG (H+L) antibody or biotinylated goat anti-Armenian hamster IgG (H+L) antibody (both Jackson ImmunoResearch Europe Ltd.). Immunohistochemical stainings of CXCL13 were performed using goat anti-rmCXCL13 polyclonal antibodies (R&D Systems, Wiesbaden, Germany) followed by biotinylated donkey anti-goat IgG (H+L) antibody (Jackson ImmunoResearch Europe Ltd.). Biotinylated antibodies were detected by alkaline phosphatase-conjugated streptavidin (DakoCytomation, Hamburg, Germany) and developed with the Vector Red Alkaline Phosphatase Substrate Kit I (Vector Laboratories, Burlingame, CA). Sections were counterstained with Meyer's hemalaun, mounted with Aquatex (Merck, Darmstadt, Germany), and analyzed by light microscopy (Axioskop 2 plus; Zeiss, Jena, Germany).

Flow Cytometry

For surface phenotyping single cell suspensions of LN cells were stained with the following fluorochrome (fluorescein isothiocyanate, phycoerythrin, or allophycocyanin)-labeled or biotinylated mAbs (all BD Biosciences): anti-CD45R (B220), anti-CD19 (ID3), anti-CD3 ϵ (145-2C11), anti-CD4 (RM4-5), anti-CD8 α (5H10), anti-CD11b (M1/70), anti-CD11c (HL3), anti-Ly-6G/C (Gr-1; RB6-8C5), anti-NK1.1 (PK136), anti-CD49b (DX5), anti-MHC class II (M5/114.15.2), anti-CD80 (16-10A1), anti-CD86 (GL1), and anti-CD40 (3/23). For the detection of biotinylated antibodies streptavidin-phycoerythrin, streptavidin-peridinin chlorophyll protein (PerCP), or streptavidin-allophycocyanin (BD Biosciences) was used. All analyses were performed on a FACS Calibur (BD Biosciences) applying the Cell Quest Pro software (BD Biosciences). Dead cells were detected by adding propidium iodide (PI, 1 μ g/ml) before analysis.

Proliferation and Migration of Cells in Vivo

Migration of cells into the LN was followed by transfer of fluorescence-labeled cells. Splenocytes of naïve C57BL/6 mice were incubated with 5- (and 6)-carboxy-

fluorescein diacetate succinimidyl ester (CFSE, 0.5 μ mol/L; Molecular Probes, Invitrogen, Karlsruhe, Germany) for 10 minutes at 37°C in PBS. Labeling of cells was stopped by the addition of fetal calf serum (5%) and two washes with PBS. Of the CFSE-labeled cells, 2×10^7 were resuspended in PBS and injected intravenously into unilaterally infected recipient mice at days 5, 10, or 18 after *Bartonella* infection, respectively. Thirteen hours after transfer popLNs were analyzed for the presence of CFSE-positive cells by flow cytometry.

To investigate cell proliferation *in vivo* the thymidine analog 5-bromo-2'-deoxyuridine (BrdU) was injected intraperitoneally to unilaterally infected and control mice at days 10 or 20 after *Bartonella* infection, respectively. Mice were sacrificed 45 minutes after injection of BrdU (1 mg/mouse) and the number of cells that incorporated BrdU into the DNA during proliferation was determined via the APC BrdU flow kit (BD Biosciences) according to the manufacturer's instructions.

Generation of Bone Marrow-Derived Conventional Dendritic Cells (BM-cDCs), Plasmacytoid Dendritic Cells (BM-pDCs), and Macrophages (BM Macrophages)

For the generation of BM-cDCs, total bone marrow (BM) cells were cultured in RPMI 1640 medium (catalog number 21875-034; Invitrogen, Carlsbad, CA) supplemented with 10 mmol/L HEPES, 0.05 mmol/L 2-mercaptoethanol (2-ME), 100 μ g/ml penicillin, 100 μ g/ml streptomycin (all from Sigma-Aldrich Taufkirchen, Germany), 10% fetal calf serum (PAA, Coelbe, Germany), and granulocyte-macrophage colony-stimulating-factor (GM-CSF) as described.^{24,25} GM-CSF-expanded BM cultures (days 7 to 10) contained 80 to 90% CD11b⁺CD11c⁺ cDCs.

For the generation of BM-pDCs, total BM cells were cultured in RPMI 1640 medium (catalog number 21875-034, Invitrogen) supplemented with 1 mmol/L sodium pyruvate (Invitrogen), 0.05 mmol/L 2-ME, 1% nonessential amino acids, 100 μ g/ml kanamycin sulfate (all from Sigma-Aldrich), 10% fetal calf serum (PAA), and 100 ng/ml recombinant murine fms-like tyrosine kinase-3 ligand (rmFlt3L, R&D Systems).²⁶ At day 7 or 8, CD11b⁻CD11c⁺CD62L⁺ BM-pDCs were purified by MoFlo (Cytomation Inc., Fort Collins, CO) sorting (purity >95%).

For the generation of BM macrophages, total BM cells were cultured in hydrophobic Teflon bags (DuPont, purchased via Cadillac Plastic, Karlsruhe, Germany) in Dulbecco's modified Eagle's medium (catalog number 41966, Invitrogen) supplemented with 0.05 mmol/L 2-ME, 1% nonessential amino acids, 10% fetal calf serum (PAA), 5% horse serum (Cell Concepts, Umkirch, Germany), and 5 ng/ml recombinant murine macrophage colony-stimulating factor (rmM-CSF, R&D Systems) for 8 days.^{27,28} All cell cultures were kept at 37°C and 5% CO₂/95% humidified air except for the Teflon bags, which were incubated with 10% CO₂/90% humidified air.

Stimulation of Myeloid Cells in Vitro

BM-cDCs, BM-pDCs, or BM macrophages were seeded into 96-well (1×10^5 cells/well, 250 μ l, analysis of culture supernatants) or 24-well plates (1×10^6 cells/well, 1 ml, analysis of mRNA expression and maturation) and stimulated at 37°C and 5% CO₂/95% humidified air using the culture media described above, with two exceptions: all media were devoid of antibiotics and specific growth factors and BM macrophages were activated in the same medium as BM-cDCs. The cells were exposed to the following stimuli for 24 to 72 hours: CpG ODN 2216 (1 μ mol/L; Thermo Electron, Ulm, Germany), lipopolysaccharide (*E. coli* O111:B4, 200 ng/ml; Sigma-Aldrich), recombinant murine interferon (rmIFN)- γ (20 ng/ml; provided by Dr. G. Adolf, Ernst Boehringer Institut, Vienna, Austria), live or inactivated *Bartonella* [multiplicity of infection (MOI) as indicated in the text].

Cytokine and Nitrite Measurements

The nitrite (NO₂⁻) content of culture supernatants was measured by the Griess assay.²⁹ IFN- α/β levels in cell culture supernatants were determined with a bioassay that is based on the protection of L929 fibroblasts against the cytopathic effect of vesicular stomatitis virus.³⁰ Briefly, triplicates of serial twofold dilutions of cell culture supernatants were preincubated with L929 fibroblasts for 24 hours at 37°C before the addition of vesicular stomatitis virus for a subsequent culture period of 48 hours. Afterward, the viability of L929 cells was measured by MTT assay.³¹ Purified mouse IFN- α/β and a neutralizing sheep anti-IFN- α/β antiserum (kindly provided by Ion Gresser, Institute Curie, Paris, France) were used as a standard and to ascertain that all antiviral activity in the supernatants was attributable to IFN- α/β , respectively. The content of IL-12p40, IL-12p70 (sensitivity 20 to 40 pg/ml, both BD Biosciences) and tumor necrosis factor (sensitivity 40 pg/ml, R&D Systems) was measured by capture enzyme-linked immunosorbent assay.

RNA Preparation, Reverse Transcriptase (RT)-PCR, and TaqMan PCR

Total RNA was prepared with the RNeasy extraction kit (Qiagen) and contaminating genomic DNA was digested using the DNA-free kit (Ambion, Austin, TX). cDNAs were generated from 1 μ g of RNA in the presence of random hexamer primers using the High Capacity cDNA archive kit (Applied Biosystems, Darmstadt, Germany) following the manufacturer's recommendations. For subsequent real-time PCR a reaction mixture (15 μ l) of TaqMan Universal Master Mix (Applied Biosystems), 20 ng of cDNA, and Assays-on-Demand (Applied Biosystems) including forward and reverse primers and the 6-FAM-labeled probe for the target gene [mIFN- α 4: Mm00833969_s1, mIFN- α 5: Mm00833976_s1, mIFN- α 9: Mm00833983_s1, mIFN- α 11: Mm01257312_s1, mIFN- α 12: Mm00616656_s1, mIFN- α 13: Mm00781548_s1, IFN- β : Mm00439546_s1,

mCXCL13: Mm00444534_m1, and mouse hypoxanthine-guanine-phosphoribosyltransferase-1 (HPRT-1): Mm00446968_m1] was prepared. PCR amplification and detection were performed on an ABI Prism 7900 sequence detector (Applied Biosystems) using the following profile: 2 minutes at 50°C, 10 minutes at 95°C, and 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. Each sample was amplified in triplicates and mRNA levels were analyzed with the SDS2.1 software (Applied Biosystems). The amount of mRNA of each gene of interest was normalized to the housekeeping gene mHPRT-1. mRNA expression levels were calculated by the following formula: relative expression = $2^{-(C_{T(\text{Target})} - C_{T(\text{mHPRT-1})})}$.

Statistics

Statistical analysis was performed with the help of the software GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA) using the two-tailed Student's *t*-test or analysis of variance with a multiple comparison post test as indicated in Results.

Results

Subcutaneous Infection of Immunocompetent Mice with B. henselae, but Not with B. grahamii Causes a Strong and Long-Lasting Regional LN Swelling

To imitate the natural route of infection, mice were subcutaneously infected with *B. henselae* Fr98/K8 or with *B. henselae* Fr98/K8aM into the dorsum of the left and/or right hind foot. Initial titration of the infection dose (10^6 to 10^9 CFU/inoculum) revealed that an inoculum of 10^7 to 10^8 CFU *B. henselae* was necessary to induce a transient local inflammation at the site of infection and a strong and long-lasting enlargement of the draining popLNs of immunocompetent C57BL/6 or BALB/c mice (Figure 1, A and B; and data not shown). Because more consistent results were obtained with an inoculum of 10^8 CFU (data not shown), we decided to use this infection dose throughout our study. The LN swelling (as quantified by weighing the LNs) reached its maximum after 3 to 4 weeks and lasted for at least 8 weeks after infection (Figure 1A). Lymphadenopathy was also observed in C57BL/6 mice subcutaneously infected with *B. henselae* grown in the absence of *S. epidermidis* (data not shown). In contrast, C57BL/6 mice that were infected with the same dose of *B. grahamii*, a rodent-specific *Bartonella* species, developed only a mild and short-lived lymphadenopathy (Figure 1A) and mice infected with various control inocula (see Material and Methods) did not show any sign of lymphadenopathy (data not shown).

Three to four weeks after infection the draining popLNs of *B. henselae*-infected mice had a significantly higher weight [median of 13.3 mg (strain Fr98/K8) and 12.6 mg (strain Fr98K8/aM)] than those of *B. grahamii*-infected mice (median weight of 6.1 mg) (Figure 1B). Accordingly,

Figure 1. Changes in the weight of draining popLNs after subcutaneous inoculation of live or inactivated *Bartonella* (1 to 4×10^8 CFU) into C57BL/6 mice. **A:** Development of LN weight from day 1 to 15 weeks after *B. henselae* (strain Fr98/K8) and from day 1 to 4 weeks after *B. grahamii* infection. Mean $\pm$ SEM of five independent experiments with three to six mice per group (unilateral infection) are shown, respectively. **B:** Comparison of the *B. henselae*- versus *B. grahamii*-induced increase of LN weights 3 to 4 weeks after infection. Results of 21 (*B. henselae* Fr98/K8aM) and 19 (*B. grahamii*) independent experiments, respectively. As a control draining popLNs after PBS injection were weighed. **C:** Influence of bacterial viability on LN weight. Live, heat-inactivated (95°C , 15 minutes) or sonicated (3×5 minutes on ice, pulsed) bacteria (*B. henselae* Fr98/K8aM, *B. grahamii*), respectively, were subcutaneously injected into mice. Three weeks after infection the weight of the draining popLNs was determined. Results of three independent experiments. **B** and **C:** Each dot in the figures represents the weight of one single draining popLN of unilaterally or bilaterally infected mice. —, median. *P* values were determined by one-way analysis of variance and Tukey-Kramer post test.

there was a 29-fold increase in the total cell number of draining popLNs from *B. henselae* (Fr98/K8aM)-infected mice [$23 (\pm 7.3) \times 10^6$ cells/LN] compared to PBS-treated controls [$0.8 (\pm 0.15) \times 10^6$ cells/LN], but only a sevenfold increase in the *B. grahamii*-infected group [$5.3 (\pm 1.0) \times 10^6$ cells/LN] (median $\pm$ SEM of six experiments). The induction of the lymphadenopathy by *Bartonella* did not require viable bacteria. In fact, heat-inactivated or sonicated *B. henselae* and *B. grahamii* elicited tentatively or even significantly larger popliteal LNs compared to untreated, living bacteria (Figure 1C). In 5 to 20% of human CSD cases atypical symptoms occur.^{10,11} In our mouse model none of the infected mice showed any alteration in behavior, general condition, or on autopsy. From these data we concluded that the LN swelling after *B. henselae* infection is a local event, which is significantly more pronounced than the *B. grahamii*-induced lymphadenopathy and which does not lead to a clinically apparent systemic illness.

Lymphadenopathy Develops Despite Rapid Elimination of Bacteria

In humans with CSD viable *B. henselae* are rarely isolated from affected LNs.⁹ In mice *B. henselae* is eliminated within a few days to 1 week after systemic (intraperitoneal or intravenous) infection.^{18–22,32} In our model of local (subcutaneous) infection, *B. henselae* could be cultured from the draining popLNs of five of six analyzed C57BL/6 mice at day 1 after infection, whereas at day 20 after infection all of the 20 or 15 analyzed C57BL/6 mice that had been inoculated with *B. henselae* Fr98/K8aM or *B. henselae* Fr98/K8, respectively, had sterile LNs. However, DNA of *B. henselae* was present in 5 of 15 analyzed popLNs at day 21 after infection (*B. henselae* Fr98/K8).

The spleen cell culture of one of six subcutaneously infected mice was positive for *B. henselae* at day 1 after infection. At day 21 after infection low numbers of viable *Bartonella* were recovered from 1 of 15 spleens and from 6 of 58 spleens of C57BL/6 mice infected with *B. henselae* Fr98/K8 or *B. henselae* Fr98/K8aM, respectively (0.5 to 49 *B. henselae* CFU/mg spleen; data not shown), which correlated with positive *Bartonella* PCR reactions (data not shown). The analysis of *B. grahamii*-infected mice yielded similar culture and PCR results (data not shown). With the exception of one mouse (which showed a positive *Bartonella* PCR reaction of the liver and blood on day 21 after infection), neither cultivable bacteria nor *Bartonella* DNA were detectable in the liver and heart blood of all 8 C57BL/6 mice analyzed at day 1 after infection or of all 15 (*B. henselae* Fr98/K8) and 33 (*B. henselae* Fr98/K8aM) C57BL/6 mice that were examined 3 weeks after infection. Together, these data indicate that a local inoculation of *B. henselae* leads to a long-lasting lymphadenopathy, which does not require persistent infection and which is only rarely associated with a systematic (but clinically inapparent) spread of the bacteria.

Increase of B cells, but No Formation of Granulomata in Draining LNs of *B. henselae*-Infected Mice

Similar to humans with CSD¹² C57BL/6 mice infected subcutaneously with *B. henselae* produced high amounts of *B. henselae*-specific IgG (serum antibody titer >1:512 at 4 to 8 weeks after infection in all 17 mice tested, data not shown). In CSD patients a high B-cell content is found within the afflicted LNs.^{10,13–15} We therefore investigated the structure and cellular composition of draining popLNs of *B. henselae*-infected C57BL/6 mice by histology. We found no necrotic areas or any kind of granuloma formation on subcutaneous *Bartonella* infection in sections stained with H&E or stained immunohistochemically for myeloid cells (CD11b⁺; F4/80⁺), granulocytes (GR-1⁺), or DCs (CD11c⁺) (Figure 2 and data not shown). Instead,

Figure 2. Histopathology of draining popLNs on *B. henselae* infection. The distribution of B220⁺, CD4⁺, CD8⁺, GR-1⁺, CD11b⁺, F4/80⁺, and CD11c⁺ cells (red) and the expression of the chemokine CXCL13 (red) within the non-draining (A) and draining (B) popLNs 3 weeks after subcutaneous *B. henselae* infection (1×10^8 CFU of *B. henselae* Fr98/K8aM) were analyzed by immunohistochemistry of cryostat sections (5 μ m) as described in the Material and Methods section. Nuclei were counterstained with Meyer's hemalaun. Scale bars = 100 μ m.

the normal follicular structure seen in naïve LNs was primarily lost because of reactive follicular hyperplasia and because of a huge number of B cells (B220⁺) [intermingled with T cells (CD4⁺, CD8⁺)] that also expanded in interfollicular areas (Figure 2). Comparable results were obtained with popLNs from *B. grahamii*-infected mice (data not shown).

Multicolor fluorescence-activated cell sorting analysis revealed that the mononuclear cells of popLNs of naïve or PBS-injected C57BL/6 mice consisted of ~70% CD3⁺ T cells and 25% CD19⁺B220⁺ B cells. This relationship was strongly shifted toward CD19⁺B220⁺ B cells in the draining, but not in the non-draining LNs after subcutaneous infection with *B. henselae* or *B. grahamii* (Figure 3, A and B). The relative increase of B cells compared to T cells was equivalent for both *Bartonella* species, but the rise of the absolute B- and T-cell numbers was larger in the case of *B. henselae* because of the much more pronounced lymphadenopathy (data not shown). The ratio of CD3⁺CD4⁺ to CD3⁺CD8⁺ T cells and the relative num-

Figure 3. Cell composition of draining popLNs after *Bartonella* infection. A and B: Three weeks after infection with either *B. henselae* Fr98/K8aM or *B. grahamii* (1 to 4×10^8 CFU) the percentage of CD3⁺ T cells and CD19⁺ B cells was investigated by flow cytometry. As a control, the popLNs draining the contralateral, PBS-injected feet of infected mice (non drain. LN) as well as popLNs of naïve mice were analyzed. A: Percentage of CD3⁺ and CD19⁺ cells within the popLN of one representative *B. henselae*-infected mouse (unilateral infection) as shown by dot blot. B: Percentage of CD3⁺ and CD19⁺ cells of *B. henselae*-infected and *B. grahamii*-infected mice and controls [mean $\pm$ SEM of six independent experiments (drain. LN; unilateral or bilateral infection) or of three independent experiments (non drain. LN; naïve mice) with two to six samples per group, respectively].

bers of all other cell types analyzed [GR-1⁺ granulocytes, CD11c⁺ DCs, CD11b⁺ myeloid cells, NK1.1⁺/DX5⁺ CD3⁻ natural killer (NK) cells, NK1.1⁺/DX5⁺CD3⁺ NKT cells] remained unaltered after infection (data not shown). We conclude that subcutaneous infection of mice with *B. henselae* not only induces a lymphadenopathy with an increase of the total number of mononuclear cells, but also skews the cellular composition toward a relative increase of the B-cell population.

Local Proliferation as Well as Migration of Cells to the LN Contribute to *B. henselae*-Induced Lymphadenopathy

Next, we addressed the question whether the *B. henselae*-induced enlargement of the B-lymphocyte compartment in the draining LNs results from an increased migration and/or proliferation of B cells. For the analysis of cell migration we transferred CFSE-labeled total splenocytes to *Bartonella*-infected mice (days 5, 10, or 18 after infection, respectively). Thirteen hours later, we determined the phenotype of the CFSE⁺ cells that had migrated into the draining popLNs. In contrast to PBS-treated control mice, in which CD3⁺ T cells predominated the pool of cells entering the popLN, approximately half of the CFSE⁺ cells migrating into the popLN of *B. henselae*- or *B. grahamii*-infected mice were CD19⁺ B cells (Figure 4A and data not shown). The total amount of cells that migrated into a draining popLN on *Bartonella* infection was not influenced by the *Bartonella* species used for infection (data not shown). CXCL13, a chemokine involved in B-cell homing to LNs,^{17,33} was readily detectable by immunohistochemical stainings within B-cell areas of draining LNs after both *B. henselae* and *B. grahamii* infection (Figure 2 and data not shown).

For the analysis of the current LN cell proliferation at certain time points after infection BrdU that is incorporated during cell mitosis was injected intraperitoneally to unilaterally infected mice on day 10 or 20 after *Bartonella* infection. To minimize the possibility of accumulation of BrdU⁺ (ie, proliferated) cells in the draining popLN as a result of cell immigration rather than local proliferation during BrdU exposure, mice were sacrificed already 45 minutes after BrdU injection. The number (percent) and phenotype of BrdU⁺ cells in draining versus nondraining popLNs were determined by flow cytometry. In *B. henselae*-infected mice the ratio of proliferated (BrdU⁺) cells calculated by dividing the percentage of BrdU⁺ cells per draining popLN through the percentage of BrdU⁺ per nondraining popLN was significantly higher than in mice infected with *B. grahamii* at day 10 of infection [ratio 13.43 (±3.51) versus 8.40 (±1.40), *P* < 0.05 as determined by one-way analysis of variance and Bonferroni post test] and tentatively higher at day 20 after infection [ratio 6.59 (±1.71) versus 4.32 (±0.96), *P* > 0.05 as determined by one-way analysis of variance and Bonferroni post test; mean ± SD of two to four independent experiments per time point with four mice per group].

Figure 4. Migration and proliferation of T and B cells after *Bartonella* infection. **A:** Influx of B and T cells into the draining popLNs. CFSE-labeled whole splenocytes (2×10^7) of naïve C57BL/6 mice were transferred to PBS-injected control mice or to C57BL/6 recipient mice infected subcutaneously with either *B. henselae* Fr98/K8aM or *B. grahamii* 10 days earlier (dose 1 to 2×10^8 CFU; unilateral infection). Thirteen hours after the transfer the composition of CFSE⁺ cells in the draining popLNs was analyzed by flow cytometry. Percentage of CD19⁺, CD3⁺, CD4⁺, and CD8⁺ cells of all CFSE⁺ cells in the draining popLNs is shown (mean ± SEM of three independent experiments with three mice per group). **B:** Proliferation of lymphocytes in the draining popLNs. C57BL/6 mice were subcutaneously injected with either *B. henselae* Fr98/K8aM or *B. grahamii* in one hind foot (dose 1 to 2×10^8 CFU) or with PBS into the contralateral hind foot (nondrain. LN). Control mice were injected bilaterally with PBS. Twenty days after infection mice were treated with BrdU (1 mg/mouse i.p.). Forty-five minutes later the cell surface phenotype of BrdU⁺ (ie, proliferated) cells within the draining popLNs was analyzed by fluorescence-activated cell sorting. Results show the percentage of proliferated (BrdU⁺) CD19⁺, CD3⁺, CD4⁺, or CD8⁺ cells within the total BrdU⁺ cell population in the draining popLNs of *Bartonella*-infected and control mice (mean ± SD of two independent experiments with four mice per group). *P* values were determined by one-way analysis of variance and Tukey-Kramer post test.

This points to a pronounced proliferation within the draining popLN especially after *B. henselae* infection. The vast majority of BrdU⁺ cells in the draining popLNs of *Bartonella*-infected mice at day 10 and day 20 after infection were B (CD19⁺) cells (Figure 4B and data not shown), whereas the few BrdU⁺ cells in the LNs from uninfected mice or in the nondraining popLNs from *Bartonella*-infected mice were equally composed of B cells, CD4⁺ T cells, and CD8⁺ T lymphocytes.

From these data we conclude that *Bartonella* infection elicits both the recruitment and the proliferation of lymphocytes (notably of B cells). These processes lead

Figure 5. *Bartonella*-induced maturation of BM-cDCs. After a 24-hour incubation of BM-cDCs with medium alone or with lipopolysaccharide (1 μ g/ml), live *B. henselae* (strain ATCC 49882, MOI 10; and Fr98/K8aM, MOI 6) or live *B. grahamii* (MOI 32), the expression of MHC class II, CD40, CD80, and CD86 on the surface of live CD11b⁺CD11c⁺ cells was determined by flow cytometry. The expression of MHC II, CD40, CD80, and CD86 is shown in gray, the antibody-isotype control staining in white. The mean fluorescence intensity is reported in each histogram. One representative of four independent experiments is shown.

to increased cell numbers in the draining LNs of infected mice. Lymphocyte proliferation, however, is differentially enhanced in *B. henselae*-infected mice compared to *B. grahamii*-infected mice, contributing to the distinct course of LN swelling in the two groups.

Bartonella Activates Conventional and Plasmacytoid DCs as Well as Macrophages

Recently, it was shown that human DCs mature and are activated on infection with *B. henselae* *in vitro*.¹⁷ In an attempt to characterize the inflammatory cytokine milieu that might orchestrate the *Bartonella*-induced lymphadenopathy in mice, we investigated the activation of BM-cDCs, BM macrophages, and BM-pDCs of C57BL/6 mice by *Bartonella*. *B. henselae* as well as *B. grahamii* induced the up-regulation of MHC class II, CD40, CD80, and CD86 on the surface of BM-cDCs, which indicates their maturation (Figure 5). Furthermore, co-cultivation of BM-cDCs or BM macrophages with *Bartonella* led to the release of nitrite (as the stable end product of inducible NO synthase activity) and of proinflammatory mediators such as tumor necrosis factor and IL-12p40 (Table 1), which are known regulators of the differentiation and prolifera-

tion of B cells.^{34,35} This cytokine response was not dependent on viable *Bartonella*, but also elicited by inactivated bacteria (data not shown).

Plasmacytoid dendritic cells (pDCs) that are exposed to bacterial DNA or synthetic oligonucleotides containing unmethylated CpG motifs, release high amounts of IFN- α/β ,^{36,37} which is known to modulate the development and differentiation of B cells.³⁸ We therefore tested *Bartonella*-stimulated BM-pDCs for the expression of type I IFN genes and the release of IFN- α/β . With the exception of IFN- α 11 and IFN- α 13 (which were induced no more than 10-fold) the mRNA expression of all other investigated IFN- α/β subtypes (IFN- α 4, IFN- α 5, IFN- α 9, IFN- α 12, and IFN- β) was up-regulated by a factor of 10^2 to 10^4 in response to *Bartonella* (Figure 6A). On the mRNA level *B. henselae* (strain ATCC 49882 and Fr98/K8aM) and *B. grahamii* led to a comparable expression of IFN- α/β (Figure 6A). On the protein level, however, *B. grahamii* always induced significantly higher amounts of IFN- α/β than *B. henselae*, especially at a MOI <1 (Figure 6B). Stimulation of pDCs with inactivated *B. henselae* or inactivated *B. grahamii* led to a significant reduced release of IFN- α/β protein compared to stimulation with the respective live bacteria (Figure 6C). In contrast to BM-pDCs, BM-cDCs and BM macrophages secreted much lower amounts of IFN- α/β (Figure 6B versus Table 1). These data demonstrate that both *B. henselae* and *B. grahamii* stimulate BM-cDCs, BM macrophages, and BM-pDCs for the release of proinflammatory and immunoregulatory cytokines. However, the production of IFN- α/β protein by BM-pDCs was significantly lower in response to *B. henselae* as compared to *B. grahamii*.

IFN- α/β Receptor Signaling Limits *Bartonella*-Induced Lymphadenopathy

The inverse relationship between IFN- α/β induction *in vitro* (Figure 6, B and C) and the degree of lymphadenopathy *in vivo* (Figure 1) suggested that a differential expression of IFN- α/β might account for the strikingly distinct course of LN swelling in *B. henselae*-infected versus *B. grahamii*-infected mice. Because all type I IFN subtypes bind to one common IFN- α/β receptor (IFNAR),^{38,39} we used IFNAR chain 1-deficient C57BL/6 mice (IFNAR1^{-/-}) to elucidate the po-

Table 1. Cytokine and Nitric Oxide Production by BM-cDCs and BM-Macrophages Exposed to *B. henselae* or *B. grahamii*

		<i>B. henselae</i>									
				ATCC 49882		Fr98/K8aM		<i>B. grahamii</i>			
Cell type	Stimulation	Control	LPS + rmlIFN- γ	MOI 1 to 10	MOI 10 to 50	MOI 1 to 10	MOI 10 to 50	MOI 1 to 10	MOI 10 to 50		
BM-cDC	Nitrite (μ mol/L)	b.d.l.	67 $\pm$ 6	1 $\pm$ 0.4	19 $\pm$ 8	24 $\pm$ 9	44 $\pm$ 11	11 $\pm$ 5	40 $\pm$ 5		
	TNF (pg/ml)	b.d.l.	6188 $\pm$ 1719	1151 $\pm$ 328	2625 $\pm$ 328	2570 $\pm$ 669	4055 $\pm$ 187	3817 $\pm$ 1105	5534 $\pm$ 2306		
	IL-12p40 (pg/ml)	1205 $\pm$ 171	8691 $\pm$ 1826	3978 $\pm$ 2306	5859 $\pm$ 1752	5541 $\pm$ 2010	7663 $\pm$ 3152	5450 $\pm$ 2090	7062 $\pm$ 2042		
	IFN- α/β (U/ml)	b.d.l.	n.d.	b.d.l.	519 $\pm$ 402	637 $\pm$ 391	1979 $\pm$ 1973	990 $\pm$ 891	2118 $\pm$ 969		
BM-M Φ	Nitrite (μ mol/L)	1 $\pm$ 0.3	73 $\pm$ 8	6 $\pm$ 4	23 $\pm$ 5	7 $\pm$ 2	27 $\pm$ 5	13 $\pm$ 4	29 $\pm$ 5		
	TNF (pg/ml)	11 $\pm$ 11	4157 $\pm$ 1292	1122 $\pm$ 482	2223 $\pm$ 497	1951 $\pm$ 751	3443 $\pm$ 774	1999 $\pm$ 274	3399 $\pm$ 799		
	IL-12p40 (pg/ml)	68 $\pm$ 39	6926 $\pm$ 1783	3417 $\pm$ 473	6702 $\pm$ 1293	3611 $\pm$ 948	4583 $\pm$ 2549	5449 $\pm$ 1865	6598 $\pm$ 1766		
	IFN- α/β (U/ml)	b.d.l.	n.d.	660 $\pm$ 389	432 $\pm$ 249	b.d.l.	193 $\pm$ 193	1153 $\pm$ 422	558 $\pm$ 263		

Cells were stimulated with either a combination of LPS (200 ng/ml) and rmlFN- γ (20 ng/ml) or with live *B. henselae* (strain ATCC 49882 or Fr98/K8aM) or *B. grahamii* (MOI 1 to 10, 10 to 50) for 24 hours (TNF) or 48 hours (nitrite, IL-12p40, IFN- α/β), respectively. As a control cells were cultured in parallel in medium without further stimuli. Values are given as the mean of three (BM-cDCs) and four (BM-macrophages) independent experiments $\pm$ SEM. b.d.l., value below detection limit; n.d., not done; BM-M Φ , BM macrophages.

Figure 6. IFN- α/β expression of murine plasmacytoid dendritic cells (BM-pDCs) exposed to live or inactivated *Bartonella*. **A:** IFN- α/β mRNA expression of sorted C57BL/6 BM-pDCs stimulated with *B. henselae* ATCC 49882, *B. henselae* Fr98/K8aM, or *B. grahamii* as indicated for 24 hours (MOI 0.5 to 3) was determined by real-time RT-PCR. Relative expression of one of three experiments is shown. **B** and **C:** IFN- α/β activity in the culture supernatants of sorted C57BL/6 BM-pDCs was measured by vesicular stomatitis virus bioassay after 48 hours of stimulation with *Bartonella* or CpG 2216 (1 μ mol/L). **B:** IFN- α/β expression of BM-pDCs on stimulation with live *B. henselae* ATCC 49882 or *B. grahamii* (MOI 0.1 to 10, as indicated; mean $\pm$ SD of 10 independent experiments with one sample per MOI and experiment, * P < 0.05 as determined by one-way analysis of variance and Tukey-Kramer post test). **C:** IFN- α/β expression of BM-pDCs after stimulation with live or heat-inactivated (95°C, 15 minutes; **left**) or with live or sonicated (3 $\times$ 5 minutes on ice, pulsed; **right**) *Bartonella* (mean $\pm$ SD of five independent experiments with one to three replicates per condition; MOI was within the range of 0.1 to 5 in the six different experimental pairs; * P < 0.05 as determined by paired Student's *t*-test).

Figure 7. *Bartonella*-induced lymphadenopathy in wild-type (wt) versus IFNARI^{-/-} mice. Three weeks after subcutaneous infection of wild-type or IFNARI^{-/-} mice with *B. henselae* Fr98/K8aM (1 $\times$ 10⁸ CFU, results of two independent experiments) or *B. grahamii* (1 to 4 $\times$ 10⁸ CFU, results of three independent experiments) the weight of the draining popLNs was determined. Each dot in the figure represents the weight of one single LN of unilaterally or bilaterally infected mice (—, median; P values were determined by one-way analysis of variance and Tukey-Kramer post test).

tential function of IFN- α/β during *Bartonella* infection *in vivo*. Three weeks after infection with *B. henselae* or *B. grahamii* no bacteria were detectable by PCR or culture in the draining popLNs, spleen, liver, and blood of wild-type and IFNARI^{-/-} mice (data not shown), indicating that IFN- α/β signaling is dispensable for the rapid elimination of *Bartonella*. IFNARI^{-/-} mice developed significantly larger LNs after infection with *B. grahamii* than the respective wild-type controls (Figure 7). In contrast, the LN swelling in *B. henselae*-infected IFNARI^{-/-} mice was not significantly different from the one seen in infected wild-type C57BL/6 mice (Figure 7). These data suggest that IFN- α/β exerts an inhibitory effect on the development of lymphadenopathy and that the weak and transient LN enlargement in *B. grahamii*-infected wild-type mice might be caused by an enhanced expression of type I IFNs.

Discussion

Although most infections of immunocompetent individuals with *B. henselae* show a benign, self-healing course,¹⁰ CSD is nevertheless a clinically highly relevant entity as reflected by its frequency,⁴⁰ the considerable spectrum of differential diagnoses,⁴¹ the occasional severe systemic manifestations,⁴² and its poorly understood pathogenesis. The lymphadenopathy of CSD is assumed to result from an immunopathogenic process,¹⁶ but until now very little is known about the underlying mechanisms because of the limitations of studying human patients. Published mouse models of *B. henselae* infection describe the course of infection after systemic (intraperitoneal or intravenous) injection. In these models, *B. henselae* bacteria were rapidly eliminated, corresponding to the fast elimination of *B. henselae* in the accidental human host.^{18–22} However, the characteristic lymphadenopathy that is seen in humans after infection with *B. henselae* has not been described in intravenously or intraperitoneally infected mice.

The Experimental Murine *B. henselae*-Infection Model of Human CSD

To the best of our knowledge, this study is the first to demonstrate that mice subcutaneously infected with *B. henselae* indeed develop a striking, regional, and long-lasting lymphadenopathy comparable to human CSD. After infection with the same dose of *B. grahamii* only mild alterations of the LN weight were detectable. This might result from the adaptation of *B. grahamii* to its natural rodent reservoir hosts^{43,44} because *B. grahamii* was shown to cause a transient bacteremia in mice after intravenous injection⁴⁵ similar to *B. henselae* in its feline reservoir.⁴⁶ It also indicates the specificity of the *B. henselae*-induced lymphadenopathy in accidental hosts. Remarkably, to the best of our knowledge *B. grahamii* was never associated with CSD in humans. Mice infected subcutaneously with *B. quintana*, a *Bartonella* species closely related to *B. henselae* and regarded as its genomic derivative,⁴⁷ also developed only a mild and short-lived lymphadenopathy (data not shown) that further supports the specificity of the *B. henselae*-induced pathology. Although high doses of *B. henselae* were necessary to elicit strong LN swellings, the bacterial dose used in our study corresponds to the dose required to induce alterations in the liver of intraperitoneally infected mice.¹⁸ With respect to the chosen high-dose inoculum it is important to keep in mind that several millions of *B. henselae* can be present in 1 ml of feline blood⁴⁸ and that cat flea feces can contain up to 10⁴ CFU *B. henselae*/mg.⁴⁹ As the cat flea and especially its feces that is present in the fur of flea-infested cats in high amounts are well accepted as transmitters for *B. henselae*, the dose of infection in human CSD might be rather high, although the exact route of infection of humans still remains to be determined. Furthermore, the inoculum that is necessary to induce a certain clinical phenotype may vary from host to host. Thus, higher inocula of *B. henselae* might be needed to elicit the typical symptoms of human CSD in mice. Related phenomena dependent on the host species have been seen in several other infection models.^{50–53} It is also quite likely that under natural conditions components of the flea feces other than *B. henselae* may enhance the development of *Bartonella*-induced lymphadenopathy and thus enable the onset of disease at a lower dose of infection in humans. Because *B. henselae* has been reported to change its phenotype during *in vivo*³² and *in vitro* passages⁵⁴ that might result in a decrease of virulence,¹⁸ we strictly used primary isolates for our infection experiments. The typical phenotype of primary isolates is characterized by small adherent colonies that tend to autoagglutinate because of the expression of an adhesin.^{54,55–57} Because of this propensity the doses of infection might slightly vary from mouse to mouse. This could be the reason for the relatively broad range of LN weights we observed after *B. henselae* infection. However, it should be noted that the course of infection and the degree of lymphadenopathy in human patients is also characterized by significant variability.^{7–9}

As seen in human CSD,^{9,12} bacteria and bacterial DNA were rapidly cleared in the draining LNs after subcutaneous application of *B. henselae* to mice. Nevertheless, the mice developed a severe lymphadenopathy that persisted far beyond the latest time points of detection of *B. henselae* in the LNs. Unexpectedly, *B. henselae* could be recultivated from the spleens of a few mice 3 weeks after subcutaneous infection. This was not seen in intravenously or intraperitoneally infected mice, in which the *Bartonella* were eliminated within 1 week,^{18–22} but is in accordance with rare cases of systemic *B. henselae* infection in immunocompetent human patients.⁵⁸ The mechanisms that cause the elimination of *B. henselae* in the LNs of all mice, but facilitate its survival in the spleen of at least some mice are currently unknown.

Mice injected with either heat-inactivated or sonicated *B. henselae* developed a striking lymphadenopathy indicating that intact protein or bacterial structures are dispensable for its induction. Surprisingly, *B. grahamii* that causes only a mild lymphadenopathy when alive, induced the most severe lymphadenopathy after sonication. This might be attributable to a higher accessibility of immunogenic bacterial components or result from the induction of inhibitory mediators (IFN- α/β) by intact *B. grahamii* as discussed below. Furthermore, the fact that different species of lysed *Bartonella* were able to induce a sterile lymphadenopathy in mice comparable to CSD in humans as well as the existence of numerous cases of human patients with clinical signs of CSD but negative serology or PCR results for *B. henselae* raises the question, whether those cases could be triggered by other members of α -proteobacteria, eg, *Rhizobium* or *Agrobacterium*. Common pathogenicity strategies within this class of bacteria have been postulated^{59,60} and besides *B. henselae* at least *Afipia felis* is also known to induce rare cases of CSD.^{61,62}

Human CSD pathology in the LN usually involves the formation of suppurative granulomata with the accumulation of B cells,^{13–15} but cases without granuloma formation were also reported depending on the time of diagnosis and the stage of infection.^{10,63} An infiltration of lymphocytes and monocytes as well as some small granulomata without central necrosis were observed in the liver of mice after intraperitoneal injection of a high dose of live but not of heat-inactivated *B. henselae*, whereas other organs were not affected.^{18,19} In contrast to these data no granuloma formation was detectable in draining LNs after subcutaneous infection with *B. henselae* that may be attributable to host species differences as already described for human versus mouse infections with *Mycobacterium tuberculosis*.⁶⁴ The histopathology of draining LNs after *B. henselae* infection in mice was characterized by a reactive follicular hyperplasia as also seen in early stages of human CSD¹⁰ and a prominent expansion of the B-cell area. Quantitative analysis confirmed an increase of B cells within draining LNs on *Bartonella* infection. The requirement of B and T cells for the development of lymphadenopathy on *Bartonella* infection is further reflected by the fact that RAG 1-deficient mice (which lack mature B and T cells⁶⁵) failed to develop a measurable lymphadenopathy after subcutaneous infec-

tion with *B. henselae* (data not shown). Together, these data clearly illustrate that the phenotypic changes (ie, the lymphadenopathy) induced by a subcutaneous infection of immunocompetent mice with *B. henselae* much more closely resemble the symptoms of human CSD than the previously published intravenous or intraperitoneal mouse infection models.

Mechanism Leading to Lymphadenopathy in Bartonella-Infected Mice

Before this study no *in vivo* data were available that explained the mechanism(s) underlying the *Bartonella*-induced lymphadenopathy. The present study demonstrates that both an altered migration and an enhanced proliferation of lymphocytes, especially of B cells, contribute to the massive and long-lasting LN swelling and the accumulation of B lymphocytes in the draining lymphoid tissue after subcutaneous *B. henselae* infection in wild-type mice.

In accordance with published data^{66–68} T-cell homing to LNs in uninfected or PBS-treated control mice was more efficient than that of B cells, whereas an enhanced recruitment of B cells into the draining LN was observed in both *B. henselae*- and *B. grahamii*-infected mice. Thus, the different extent of lymphadenopathy in response to both *Bartonella* species cannot be explained by varying induction of lymphocyte recruitment. Most likely, the increased B-cell migration is mediated by the chemokine CXCL13, which is known as the most effective B-cell chemoattractant.^{69,70} A recent study demonstrated that CXCL13-producing DCs are present in B-cell-rich human CSD granulomata *in vivo*.¹⁷ In accordance with this report we found a prominent CXCL13 protein expression within the B-cell areas of the enlarged draining LNs of *B. henselae*- or *B. grahamii*-infected mice as shown by immunohistochemistry. *In vitro*, there was 10-fold up-regulation of CXCL13 mRNA in C57BL/6 BM macrophages and BM-pDCs after exposure to *Bartonella*, no matter which *Bartonella* species (*B. henselae* or *B. grahamii*) was used (data not shown).

The analysis of lymphocyte proliferation by BrdU assays at later time points of infection (days 10 and 20) revealed a significantly (day 10 after infection) or tentatively higher (day 20 after infection) lymphocyte division rate in *B. henselae*-infected mice compared to *B. grahamii*-infected animals, which forms an additional mechanism for the CSD-like long-lasting LN swelling in response to *B. henselae*. Among the proliferating lymphocytes B cells showed by far the highest rate of cell division, thereby contributing to the modified cellular composition of draining LNs. At present the molecules and receptors that trigger this increased B-cell proliferation in response to *B. henselae* and finally lead to the sustained CSD-like lymphadenopathy are primarily unknown, but our results indicate that (1) B-cell receptor stimulation by *Bartonella* antigen is probably not involved because bacteria in the draining LNs are completely eliminated within a few days after infection and that (2) type I interferons negatively regulate the development of lymphadenopathy. A large

number of microbes and microbial products have been described to trigger the release of IFN- α/β by various cell types *in vitro* and *in vivo*.³⁸ In endothelial cells *B. henselae* induced the mRNA expression of IFN- α/β response genes, but no secretion of IFN- α/β protein.⁷¹ In the present study, BM-cDCs and BM macrophages secreted small and BM-pDCs large quantities of IFN- α/β protein *in vitro* after exposure to live *Bartonella*. Both *Bartonella* species stimulated BM-pDCs in a dose-dependent manner, but *B. grahamii* triggered the release of much higher quantities of IFN- α/β than *B. henselae*, particularly at low MOI rates. Inactivation of the bacteria, especially sonication, before BM-pDC stimulation reduced the release of IFN- α/β . In wild-type mice only sonicated, but not intact and viable *B. grahamii* induced lymphadenopathy, whereas in IFN- α/β receptor-deficient mice (IFNARI^{-/-}) also live *B. grahamii* were able to induce a CSD-like regional lymphadenopathy. Together, these data are compatible with the hypothesis that high IFN- α/β production induced by viable *B. grahamii* inhibits sustained proliferation of B lymphocytes and thus blocks the development of lymphadenopathy as observed in the case of a subcutaneous infection with *B. henselae*. Type I interferons are cytokines with pleiotropic effects and various immunoregulatory functions during infections.³⁸ With respect to the modulation of cell proliferation IFN- α/β was shown to exert anti-proliferative effects on T cells,^{72–74} but also to promote T-cell proliferation during infection.^{75–78} B cells are also known targets of type I interferons: IFN- α/β was reported to inhibit B cell lymphopoiesis,⁷⁹ to induce B cells in a state of partial activation,⁸⁰ to promote plasma cell differentiation,⁸¹ and to stimulate the antibody response and the isotype switching in B cells in response to antigens.^{82–84} Furthermore, the production of B-cell-activating factor by epithelial cells or DCs was found to be induced by IFN- β or IFN- α .^{85,86} The present study provides the first evidence that IFN- α/β influences the development of *Bartonella*-induced lymphadenopathy.

B. henselae is known as a rather inert bacterium that can cause persistent asymptomatic bacteremia despite its Gram-negative attributes.⁴⁶ *B. henselae* lipopolysaccharide activates Toll-like receptor 4 signaling only to a low extent.⁸⁷ However, both human¹⁷ and mouse DCs (Figure 5) mature on co-cultivation with *Bartonella*, and macrophages as well as DCs release proinflammatory mediators (tumor necrosis factor, IL-12p40) in response to *Bartonella*. This indicates that cells of the innate immune system are indeed able to react to *Bartonella*. Tumor necrosis factor and IL-12, which are known regulators of the differentiation and proliferation of B cells,^{34,35} may also contribute to the adaptive immune response and the accumulation of B cells in the draining LNs during the course of *Bartonella* infection. The function and antigen specificity of the expanded B-cell population in our mouse model of human CSD remain to be clarified. Human B cells within CSD granulomata were identified as monocytoid B cells expressing the transcription factor T-bet, which can mediate germinal center-independent Ig class switch in B cells.⁸⁸

In conclusion, we have shown that the subcutaneous inoculation of immunocompetent wild-type mice with *B.*

henselae leads to a CSD-like lymphadenopathy, which for the first time provides a model to study the (immuno) pathogenesis of the most frequent manifestation of human CSD. We observed that altered immune cell recruitment and an enhanced B-lymphocyte proliferation account for the *B. henselae*-induced lymphadenopathy in mice. Furthermore, mouse innate immune cells were activated by *Bartonella* *in vitro* and especially BM-pDCs secreted large quantities of IFN- α/β protein on exposure to live *Bartonella*. *In vivo*, IFN- α/β receptor signaling limits *Bartonella*-induced lymphadenopathy.

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References

- Debré R, Lamy M, Jammet ML, Costil L, Mozziconacci P: Cat scratch disease. *Sem Hop* 1950, 26:1895–1904
- Kordick DL, Breitschwerdt EB: Intraerythrocytic presence of *Bartonella henselae*. *J Clin Microbiol* 1995, 33:1655–1656
- Rolain JM, La Scola B, Liang Z, Davoust B, Raoult D: Immunofluorescent detection of intraerythrocytic *Bartonella henselae* in naturally infected cats. *J Clin Microbiol* 2001, 39:2978–2980
- Dehio C: *Bartonella*-host-cell interactions and vascular tumour formation. *Nat Rev Microbiol* 2005, 3:621–631
- Slater LN, Welch DF, Min KW: *Rochalimaea henselae* causes bacillary angiomatosis and peliosis hepatis. *Arch Intern Med* 1992, 152:602–606
- Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS: The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. *N Engl J Med* 1990, 323:1573–1580
- Dolan MJ, Wong MT, Regnery RL, Jorgensen JH, Garcia M, Peters J, Drehner D: Syndrome of *Rochalimaea henselae* adenitis suggesting cat scratch disease. *Ann Intern Med* 1993, 118:331–336
- Anderson B, Sims K, Regnery R, Robinson L, Schmidt MJ, Goral S, Hager C, Edwards K: Detection of *Rochalimaea henselae* DNA in specimens from cat scratch disease patients by PCR. *J Clin Microbiol* 1994, 32:942–948
- Fournier PE, Robson J, Zeaiter Z, McDougall R, Byrne S, Raoult D: Improved culture from lymph nodes of patients with cat scratch disease and genotypic characterization of *Bartonella henselae* isolates in Australia. *J Clin Microbiol* 2002, 40:3620–3624
- Carithers HA: Cat-scratch disease. An overview based on a study of 1,200 patients. *Am J Dis Child* 1985, 139:1124–1133
- Margileth AM: Cat scratch disease. *Adv Pediatr Infect Dis* 1993, 8:1–21
- Agan BK, Dolan MJ: Laboratory diagnosis of *Bartonella* infections. *Clin Lab Med* 2002, 22:937–962
- Facchetti F, Agostini C, Chilosi M, Mombello A, Grigolato P, Van den Oord JJ: Suppurative granulomatous lymphadenitis. Immunohistochemical evidence for a B-cell-associated granuloma. *Am J Surg Pathol* 1992, 16:955–961
- Kojima M, Nakamura S, Hosomura Y, Shimizu K, Kurabayashi Y, Itoh H, Yoshida K, Ohno Y, Kaneko A, Asano S: Abscess-forming granulomatous lymphadenitis: histological typing of suppurative granulomas and clinicopathological findings with special reference to cat scratch disease. *Acta Pathol Jpn* 1993, 43:11–17
- Kojima M, Nakamura S, Kurabayashi Y, Itoh H, Yoshida K, Asano S, Suchi T, Yoshita T: Suppurative lesions without prominent epithelioid cell response in abscess-forming granulomatous lymphadenitis. *Pathol Res Pract* 1995, 191:1072–1077
- Resto-Ruiz S, Burgess A, Anderson BE: The role of the host immune response in pathogenesis of *Bartonella henselae*. *DNA Cell Biol* 2003, 22:431–440
- Vermi W, Facchetti F, Riboldi E, Heine H, Scutera S, Stornello S, Ravarino D, Cappello P, Giovarelli M, Badolato R, Zucca M, Gentili F, Chilosi M, Doglioni C, Ponzi AN, Sozzani S, Musso T: Role of dendritic cell-derived CXCL13 in the pathogenesis of *Bartonella henselae* B-rich granuloma. *Blood* 2006, 107:454–462
- Regnath T, Mielke ME, Arvand M, Hahn H: Murine model of *Bartonella henselae* infection in the immunocompetent host. *Infect Immun* 1998, 66:5534–5536
- Arvand M, Ignatius R, Regnath T, Hahn H, Mielke ME: *Bartonella henselae*-specific cell-mediated immune responses display a predominantly Th1 phenotype in experimentally infected C57BL/6 mice. *Infect Immun* 2001, 69:6427–6433
- Kabeya H, Tsunoda E, Maruyama S, Mikami T: Immune responses of immunocompetent and immunocompromised mice experimentally infected with *Bartonella henselae*. *J Vet Med Sci* 2003, 65:479–484
- Karem KL, Dubois KA, McGill SL, Regnery RL: Characterization of *Bartonella henselae*-specific immunity in BALB/c mice. *Immunology* 1999, 97:352–358
- Velho PS, Moraes AM, Cintra ML, Giglioli R, Goncalves SA, Shlessarenko N, Camargo ME: Bacillary angiomatosis: negative results using normal Balb/c and Balb/c nude mice. *Braz J Infect Dis* 1998, 2:300–303
- Cobbald SP, Jayasuriya A, Nash A, Prospero TD, Waldmann H: Therapy with monoclonal antibodies by elimination of T-cell subsets *in vivo*. *Nature* 1984, 312:548–551
- Zal T, Volkmann A, Stockinger B: Mechanisms of tolerance induction in major histocompatibility complex class II-restricted T cells specific for a blood-borne self-antigen. *J Exp Med* 1994, 180:2089–2099
- Lutz MB, Kukutsch N, Ogilvie AL, Rossner S, Koch F, Romani N, Schuler G: An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J Immunol Methods* 1999, 223:77–92
- Gilliet M, Boonstra A, Paturel C, Antonenko S, Xu XL, Trinchieri G, O'Garra A, Liu YJ: The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. *J Exp Med* 2002, 195:953–958
- Munder M, Eichmann K, Modolell M: Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4⁺ T cells correlates with Th1/Th2 phenotype. *J Immunol* 1998, 160:5347–5354
- Schleicher U, Hesse A, Bogdan C: Minute numbers of contaminant CD8⁺ T cells or CD11b⁺CD11c⁺ NK cells are the source of IFN- γ in IL-12/IL-18-stimulated mouse macrophage populations. *Blood* 2005, 105:1319–1328
- Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem* 1982, 126:131–138
- Diefenbach A, Schindler H, Donhauser N, Lorenz E, Laskay T, MacMicking J, Rollinghoff M, Gresser I, Bogdan C: Type 1 interferon (IFN α/β) and type 2 nitric oxide synthase regulate the innate immune response to a protozoan parasite. *Immunity* 1998, 8:77–87
- Tada H, Shiho O, Kuroshima K, Koyama M, Tsukamoto K: An improved colorimetric assay for interleukin 2. *J Immunol Methods* 1986, 93:157–165
- Velho PE, de Moraes AM, Uthida-Tanaka AM, Cintra ML, Giglioli R: Ultrastructural changes in a standard strain of *Bartonella henselae* after passages through BALB/cAn mice. *Ultrastruct Pathol* 2002, 26:161–169
- Ansel KM, Ngo VN, Hyman PL, Luther SA, Forster R, Sedgwick JD, Browning JL, Lipp M, Cyster JG: A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* 2000, 406:309–314
- Jelinek DF, Lipsky PE: Enhancement of human B cell proliferation and differentiation by tumor necrosis factor- α and interleukin 1. *J Immunol* 1987, 139:2970–2976
- Dubois B, Barthelemy C, Durand I, Liu YJ, Caux C, Briere F: Toward a role of dendritic cells in the germinal center reaction: triggering of B cell proliferation and isotype switching. *J Immunol* 1999, 162:3428–3436
- Wagner H: The immunobiology of the TLR9 subfamily. *Trends Immunol* 2004, 25:381–386

37. Colonna M, Trinchieri G, Liu YY: Plasmacytoid dendritic cells in immunity. *Nat Immunol* 2004, 5:1219–1226
38. Bogdan C, Mattner J, Schleicher U: The role of type I interferons in non-viral infections. *Immunol Rev* 2004, 202:33–48
39. van Pesch V, Lanaya H, Renaud JC, Michiels T: Characterization of the murine alpha interferon gene family. *J Virol* 2004, 78:8219–8228
40. Jackson LA, Perkins BA, Wenger JD: Cat scratch disease in the United States: an analysis of three national databases. *Am J Public Health* 1993, 83:1707–1711
41. Leung AK, Robson WL: Childhood cervical lymphadenopathy. *J Pediatr Health Care* 2004, 18:3–7
42. Abdel-Haq N, Abuhammour W, Al-Tatari H, Asmar B: Disseminated cat scratch disease with vertebral osteomyelitis and epidural abscess. *South Med J* 2005, 98:1142–1145
43. Birtles RJ, Harrison TG, Saunders NA, Molyneux DH: Proposals to unify the genera *Grahamella* and *Bartonella*, with descriptions of *Bartonella talpae* comb. nov. *Bartonella peromysci* comb nov, and three new species, *Bartonella grahamii* sp nov, *Bartonella taylorii* sp nov, and *Bartonella doshaiae* sp nov. *Int J Syst Bacteriol* 1995, 45:1–8
44. Birtles RJ, Harrison TG, Molyneux DH: *Grahamella* in small woodland mammals in the U.K.: isolation, prevalence and host specificity. *Ann Trop Med Parasitol* 1994, 88:317–327
45. Koesling J, Aebischer T, Falch C, Schulein R, Dehio C: Cutting edge: antibody-mediated cessation of hemotropic infection by the intraerythrocytic mouse pathogen *Bartonella grahamii*. *J Immunol* 2001, 167:11–14
46. Chomel BB, Kasten RW, Sykes JE, Boulouis HJ, Breitschwerdt EB: Clinical impact of persistent *Bartonella* bacteremia in humans and animals. *Ann NY Acad Sci* 2003, 990:267–278
47. Alsmark CM, Frank AC, Karlberg EO, Legault BA, Ardell DH, Canback B, Eriksson AS, Naslund AK, Handley SA, Huvel M, La Scola B, Holmberg M, Andersson SG: The louse-borne human pathogen *Bartonella quintana* is a genomic derivative of the zoonotic agent *Bartonella henselae*. *Proc Natl Acad Sci USA* 2004, 101:9716–9721
48. Chomel BB, Kasten RW, Floyd-Hawkins K, Chi B, Yamamoto K, Roberts-Wilson J, Gurfield AN, Abbott RC, Pedersen NC, Koehler JE: Experimental transmission of *Bartonella henselae* by the cat flea. *J Clin Microbiol* 1996, 34:1952–1956
49. Finkelstein JL, Brown TP, O'Reilly KL, Wedincamp J Jr, Foil LD: Studies on the growth of *Bartonella henselae* in the cat flea (*Siphonaptera: pulicidae*). *J Med Entomol* 2002, 39:915–919
50. Brieland J, Freeman P, Kunkel R, Chrisp C, Hurley M, Fantone J, Engleberg C: Replicative *Legionella pneumophila* lung infection in intratracheally inoculated A/J mice. A murine model of human Legionnaires' disease. *Am J Pathol* 1994, 145:1537–1546
51. Steere AC, Coburn J, Glickstein L: The emergence of Lyme disease. *J Clin Invest* 2004, 113:1093–1101
52. Scott GH, Williams JC, Stephenson EH: Animal models in Q fever: pathological responses of inbred mice to phase I *Coxiella burnetii*. *J Gen Microbiol* 1987, 133:691–700
53. Lyons CR, Wu TH: Animal models of *Francisella tularensis* infection. *Ann NY Acad Sci* 2007, 1105:238–265
54. Kyme P, Dillon B, Iredell J: Phase variation in *Bartonella henselae*. *Microbiology* 2003, 149:621–629
55. Batterman HJ, Peek JA, Loutit JS, Falkow S, Tompkins LS: *Bartonella henselae* and *Bartonella quintana* adherence to and entry into cultured human epithelial cells. *Infect Immun* 1995, 63:4553–4556
56. Riess T, Andersson SG, Lupas A, Schaller M, Schafer A, Kyme P, Martin J, Walzlein JH, Ehehalt U, Lindroos H, Schirle M, Nordheim A, Autenrieth IB, Kempf VA: *Bartonella* adhesin A mediates a proangiogenic host cell response. *J Exp Med* 2004, 200:1267–1278
57. Riess T, Raddatz G, Linke D, Schafer A, Kempf VA: Analysis of *Bartonella* adhesin A expression reveals differences between various *B. henselae* strains. *Infect Immun* 2007, 75:35–43
58. Tappero JW, Koehler JE, Berger TG, Cockerell CJ, Lee TH, Busch MP, Stites DP, Mohle-Boetani J, Reingold AL, LeBoit PE: Bacillary angiomatosis and bacillary splenitis in immunocompetent adults. *Ann Intern Med* 1993, 118:363–365
59. Andersson SG, Kempf VA: Host cell modulation by human, animal and plant pathogens. *Int J Med Microbiol* 2004, 293:463–470
60. Kempf VA, Hitziger N, Riess T, Autenrieth IB: Do plant and human pathogens have a common pathogenicity strategy? *Trends Microbiol* 2002, 10:269–275
61. Giladi M, Avidor B, Kletter Y, Abulafia S, Slater LN, Welch DF, Brenner DJ, Steigerwalt AG, Whitney AM, Ephros M: Cat scratch disease: the rare role of *Afipia felis*. *J Clin Microbiol* 1998, 36:2499–2502
62. Del Prete R, Fumarola D, Fumarola L, Miragliotta G: Detection of *Bartonella henselae* and *Afipia felis* DNA by polymerase chain reaction in specimens from patients with cat scratch disease. *Eur J Clin Microbiol Infect Dis* 2000, 19:964–967
63. Wear DJ, Margileth AM, Hadfield TL, Fischer GW, Schlagel CJ, King FM: Cat scratch disease: a bacterial infection. *Science* 1983, 221:1403–1405
64. Pozos TC, Ramakrishnan L: New models for the study of Mycobacterium-host interactions. *Curr Opin Immunol* 2004, 16:499–505
65. Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE: RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* 1992, 68:869–877
66. Chen Q, Fisher DT, Clancy KA, Gauguier JM, Wang WC, Unger E, Rose-John S, von Andrian UH, Baumann H, Evans SS: Fever-range thermal stress promotes lymphocyte trafficking across high endothelial venules via an interleukin 6 trans-signaling mechanism. *Nat Immunol* 2006, 7:1299–1308
67. Stevens SK, Weissman IL, Butcher EC: Differences in the migration of B and T lymphocytes: organ-selective localization in vivo and the role of lymphocyte-endothelial cell recognition. *J Immunol* 1982, 128:844–851
68. Tang ML, Steeber DA, Zhang XQ, Tedder TF: Intrinsic differences in L-selectin expression levels affect T and B lymphocyte subset-specific recirculation pathways. *J Immunol* 1998, 160:5113–5121
69. Gunn MD, Ngo VN, Ansel KM, Eklund EH, Cyster JG, Williams LT: A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. *Nature* 1998, 391:799–803
70. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B: B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med* 1998, 187:655–660
71. Dehio M, Quebatte M, Foser S, Certa U: The transcriptional response of human endothelial cells to infection with *Bartonella henselae* is dominated by genes controlling innate immune responses, cell cycle, and vascular remodelling. *Thromb Haemostasis* 2005, 94:347–361
72. Sun S, Zhang X, Tough DF, Sprent J: Type I interferon-mediated stimulation of T cells by CpG DNA. *J Exp Med* 1998, 188:2335–2342
73. McNally JM, Zarozinski CC, Lin MY, Brehm MA, Chen HD, Welsh RM: Attrition of bystander CD8 T cells during virus-induced T-cell and interferon responses. *J Virol* 2001, 75:5965–5976
74. Petricoin III EF, Ito S, Williams BL, Audet S, Stancato LF, Gamero A, Clouse K, Grimley P, Weiss A, Beeler J, Finbloom DS, Shores EW, Abraham R, Larner AC: Antiproliferative action of interferon-alpha requires components of T-cell-receptor signalling. *Nature* 1997, 390:629–632
75. Aichele P, Unsoeld H, Koschella M, Schweier O, Kalinke U, Vucikuj S: Cutting edge: CD8 T cells specific for lymphocytic choriomeningitis virus require type I IFN receptor for clonal expansion. *J Immunol* 2006, 176:4525–4529
76. Curtsinger JM, Valenzuela JO, Agarwal P, Lins D, Mescher MF: Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation. *J Immunol* 2005, 174:4465–4469
77. Feng H, Zhang D, Palliser D, Zhu P, Cai S, Schlesinger A, Maliszewski L, Lieberman J: Listeria-infected myeloid dendritic cells produce IFN-beta, priming T cell activation. *J Immunol* 2005, 175:421–432
78. Ogasawara K, Hida S, Weng Y, Saiura A, Sato K, Takayanagi H, Sakaguchi S, Yokochi T, Kodama T, Naitoh M, De Martino JA, Taniguchi T: Requirement of the IFN-alpha/beta-induced CXCR3 chemokine signalling for CD8+ T cell activation. *Genes Cells* 2002, 7:309–320
79. Gongora R, Stephan RP, Zhang Z, Cooper MD: An essential role for Daxx in the inhibition of B lymphopoiesis by type I interferons. *Immunity* 2001, 14:727–737
80. Braun D, Caramalho I, Demengeot J: IFN-alpha/beta enhances BCR-dependent B cell responses. *Int Immunol* 2002, 14:411–419
81. Jego G, Palucka AK, Blanck JP, Chalouni C, Paschereau J: Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* 2003, 19: 225–234
82. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF: Type I interferons potentially enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. *Immunity* 2001, 14:461–470

83. Le Bon A, Thompson C, Kamphuis E, Durand V, Rossmann C, Kalinke U, Tough DF: Cutting edge: enhancement of antibody responses through direct stimulation of B and T cells by type I IFN. *J Immunol* 2006, 176:2074–2078
84. Zhu J, Huang X, Yang Y: Type I IFN signaling on both B and CD4 T cells is required for protective antibody response to adenovirus. *J Immunol* 2007, 178:3505–3510
85. Kato A, Truong-Tran AQ, Scott AL, Matsumoto K, Schleimer RP: Airway epithelial cells produce B cell-activating factor of TNF family by an IFN-beta-dependent mechanism. *J Immunol* 2006, 177: 7164–7172
86. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, Cerutti A: DCs induce CD40-independent immunoglobulin class switching through BLYS and APRIL. *Nat Immunol* 2002, 3:822–829
87. Zähringer U, Lindner B, Knirel YA, van den Akker WM, Hiestand R, Heine H, Dehio C: Structure and biological activity of the short-chain lipopolysaccharide from *Bartonella henselae* ATCC 49882T. *J Biol Chem* 2004, 279:21046–21054
88. Peng SL, Szabo SJ, Glimcher LH: T-bet regulates IgG class switching and pathogenic autoantibody production. *Proc Natl Acad Sci USA* 2002, 99:5545–5550