1-2008

Phagocytosis of Borrelia burgdorferi, the Lyme Disease Spirochete, Potentiates Innate Immune Activation and Induces Apoptosis in Human Monocytes

Adriana R. Cruz
University of Connecticut School of Medicine and Dentistry

Meagan W. Moore
University of Connecticut School of Medicine and Dentistry

Carson J. La Vake
University of Connecticut School of Medicine and Dentistry

Christian H. Eggers
University of Connecticut School of Medicine and Dentistry

Juan C. Salazar
University of Connecticut School of Medicine and Dentistry

See next page for additional authors

INFECTION AND IMMUNITY, Jan. 2008, p. 56–70 Vol. 76, No. 1
0019-9567/08/$08.00+0 doi:10.1128/IAI.01039-07

Phagocytosis of *Borrelia burgdorferi*, the Lyme Disease Spirochete, Potentiates Innate Immune Activation and Induces Apoptosis in Human Monocytes[▽]

Adriana R. Cruz,[1]†‡ Meagan W. Moore,[1]† Carson J. La Vake,[1] Christian H. Eggers,[1]§ Juan C. Salazar,[2] and Justin D. Radolf[1,3]*

Departments of Medicine[1] and Genetics and Developmental Biology,[3] University of Connecticut Health Center, Farmington, Connecticut 06030-3715, and Department of Pediatrics, Division of Infectious Diseases, Connecticut Children's Medical Center, Hartford, Connecticut 06106[2]

Received 27 July 2007/Returned for modification 7 September 2007/Accepted 8 October 2007

We have previously demonstrated that phagocytosed *Borrelia burgdorferi* induces activation programs in human peripheral blood mononuclear cells that differ qualitatively and quantitatively from those evoked by equivalent lipoprotein-rich lysates. Here we report that ingested *B. burgdorferi* induces significantly greater transcription of proinflammatory cytokine genes than do lysates and that live *B. burgdorferi*, but not *B. burgdorferi* lysate, is avidly internalized by monocytes, where the bacteria are completely degraded within phagolysosomes. In the course of these experiments, we discovered that live *B. burgdorferi* also induced a dose-dependent decrease in monocytes but not a decrease in dendritic cells or T cells and that the monocyte population displayed morphological and biochemical hallmarks of apoptosis. Particularly noteworthy was the finding that apoptotic changes occurred predominantly in monocytes that had internalized spirochetes. Abrogation of phagocytosis with cytochalasin D prevented the death response. Heat-killed *B. burgdorferi*, which was internalized as well as live organisms, induced a similar degree of apoptosis of monocytes but markedly less cytokine production. Surprisingly, opsonophagocytosis of *Treponema pallidum* did not elicit a discernible cell death response. Our combined results demonstrate that *B. burgdorferi* confined to phagolysosomes is a potent inducer of cytosolic signals that result in (i) production of NF-κB-dependent cytokines, (ii) assembly of the inflammasome and activation of caspase-1, and (iii) induction of programmed cell death. We propose that inflammation and apoptosis represent mutually reinforcing components of the immunologic arsenal that the host mobilizes to defend itself against infection with Lyme disease spirochetes.

Lyme disease (LD), the most prevalent arthropod-borne disease in the United States, is an infectious disorder caused by the tick-transmitted spirochetal pathogen *Borrelia burgdorferi* (108, 109). LD is usually heralded by erythema migrans, an expanding annular rash which develops in the majority of patients following the inoculation of spirochetes into skin by feeding ticks (90, 108). Dissemination of spirochetes from the site of inoculation may give rise to clinical manifestations typically involving the skin, joints, peripheral and central nervous systems, or heart (108). Constitutional symptoms frequently accompany infection, even in patients with ostensibly localized disease (90, 108), and may persist for variable periods despite adequate treatment (11, 69). In a small percentage of individuals, joint involvement may progress to a chronic or recurrent oligoarthritis that is refractory to antimicrobial therapy (110, 111).

Genomic sequencing has revealed that *B. burgdorferi* lacks orthologs of known exotoxins, as well as the specialized machinery required for the delivery of noxious molecules into host cells (26, 46). Consequently, it is now widely believed that spirochete constituents trigger local and systemic immune processes that damage tissues but also promote bacterial clearance and limit dissemination (110, 124). Consistent with this notion is the impressive capacity of *B. burgdorferi* to elicit the production of diverse inflammatory mediators in vitro (14, 29, 34, 49, 58). There also is an extensive body of evidence implicating this bacterium's abundant lipid-modified proteins, which signal via CD14 and/or Toll-like receptor 2 (TLR2)/TLR1 heterodimers (3, 7, 9, 18, 50, 60, 100, 105, 106, 123), as major proinflammatory agonists during infection. Recognition that the host needs to protect itself against uncontrolled inflammation has spawned interest in how LD spirochetes might down-modulate inflammatory processes; such studies have focused mainly on the ability of spirochetes and spirochetal products to induce the production of the anti-inflammatory cytokine interleukin-10 (IL-10) (19, 36, 42).

Investigations of innate responses to bacterial pathogens have relied heavily upon the use of monocytic or transformed nonmyeloid cell lines in conjunction with isolated or synthetic microbial molecular patterns (3). Despite their unquestioned utility for dissecting innate signaling pathways, such approaches are likely to yield an incomplete picture of how structurally complex, live bacteria are recognized by the innate immune system. Recently, we described an ex vivo system in

* Corresponding author. Mailing address: University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3715. Phone: (860) 679-8480. Fax: (860) 679-1358. E-mail: JRadolf@up.uchc.edu.

† A.R.C. and M.W.M. contributed equally to this research.

‡ Present address: Centro Internacional de Entenamiento e Investigaciones, Cali, Colombia.

§ Present address: Department of Biomedical Sciences, Quinnipiac University, Hamden, CT 06518.

▽ Published ahead of print on 15 October 2007.

which freshly isolated peripheral blood mononuclear cells (PBMCs) are used to examine the interactions of pathogenic spirochetes with the immune cells that *B. burgdorferi* is likely to encounter during the early dissemination phase of human LD (88). With this model, we found that phagocytosed *B. burgdorferi* initiated activation programs in monocytes and dendritic cells (DCs) that differed qualitatively and quantitatively from those evoked by equivalent lipoprotein-rich lysates. The markedly enhanced secretion of IL-1β by monocytes incubated with live spirochetes was particularly significant because it indicated that phagocytosed *B. burgdorferi* has the capacity to generate a cytosolic signal for the assembly of the inflammasome, a multiprotein complex which cleaves procaspase-1 to the active enzyme; activated caspase-1 is an absolute prerequisite for the processing and secretion of IL-1β (80, 84). In contrast to *B. burgdorferi*, intracellular pathogens generate the cytosolic danger signal for activation of caspase-1 by escaping from the phagosome or by secreting molecules that interact with pathogen sensors in the cytosol (3, 80, 84).

In the present study, we demonstrated that phagocytosis of live *B. burgdorferi* not only results in an enhanced inflammatory response but also induces apoptosis in monocytes. Surprisingly, in light of reports describing the proapoptotic properties of bacterial lipoproteins (8, 9, 10, 62, 78), we did not observe a cell death response in PBMCs incubated with equivalent spirochetal lysates. Two findings indicated that the inflammatory and death responses elicited by phagocytosed LD spirochetes involve distinct signaling pathways: (i) maximal production of cytokines required internalization of viable *B. burgdorferi*, whereas heat-killed and live spirochetes were comparably efficient at promoting cell death; and (ii) opsonophagocytosis of the syphilis spirochete, *Treponema pallidum*, which is highly stimulatory under the same conditions (88), failed to trigger monocyte cell death. Collectively, our results further underscored the ability of LD spirochetes within phagolysosomes to induce cytosolic signals that differ from those generated when spirochetal constituents engage cell surface pattern recognition receptors. They also led us to propose that inflammation and apoptosis represent mutually reinforcing components of the immunologic arsenal that the host mobilizes to defend itself against infection with LD spirochetes.

MATERIALS AND METHODS

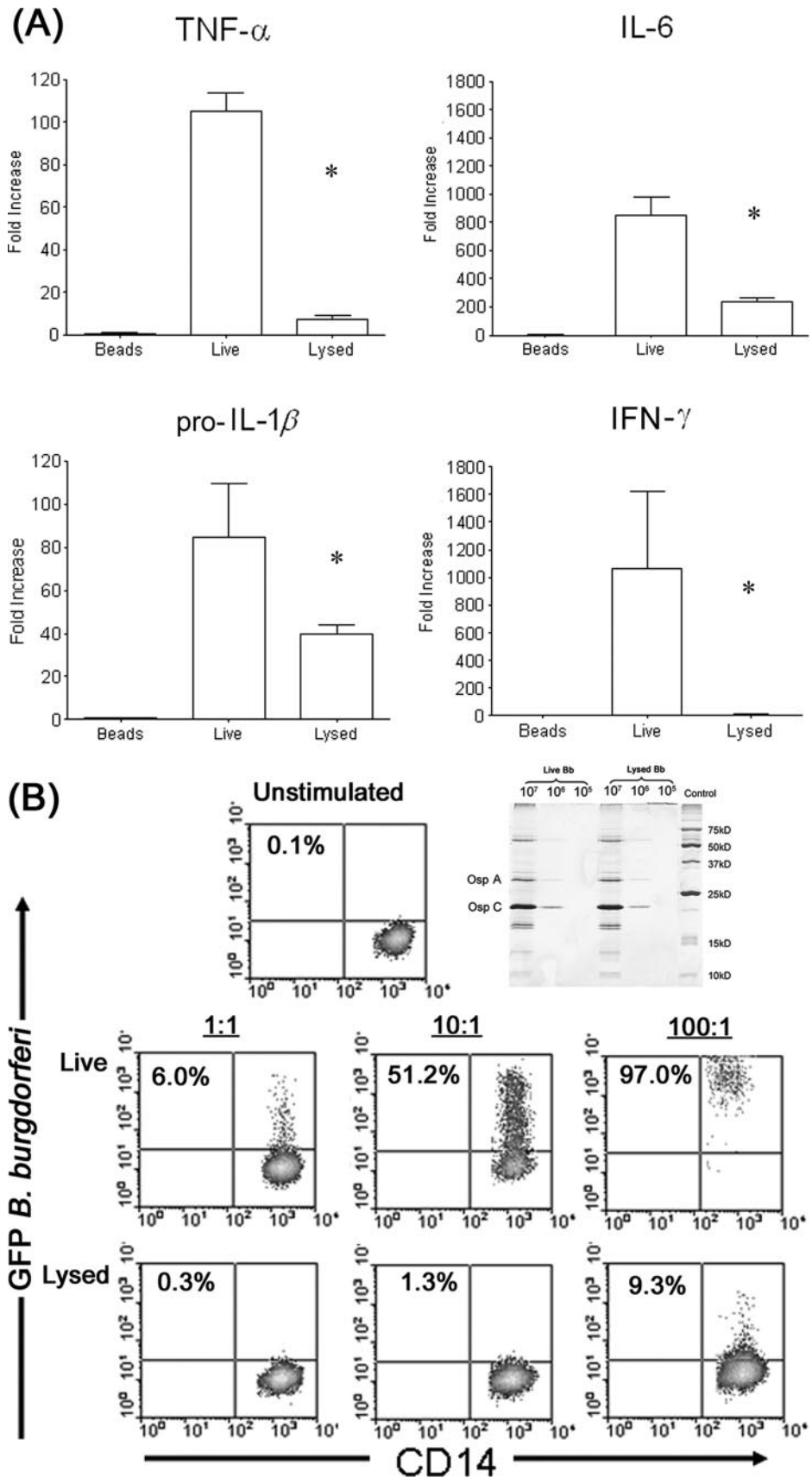
Human subjects. The University of Connecticut Health Center (UCHC) Institutional Review Board approved all protocols used in this study. Healthy volunteers with no history of syphilis or LD were recruited by the General Clinical Research Center at UCHC. After written informed consent was obtained, blood was collected by venipuncture. Volunteers were confirmed to be seronegative for LD or syphilis by serological tests conducted in the UCHC clinical laboratory.

Bacteria and bacterial preparations. Low-passage *B. burgdorferi* 297 and Bb-GFP, a virulent *B. burgdorferi* 297 clone transformed with a pCE320 derivative harboring a transcriptional fusion of the green fluorescent protein (GFP) gene (*gfp*) and the constitutive *flaB* promoter (*gfp*-P_{flaB}) (39, 40), were propagated in Barbour-Stoenner-Kelly medium containing 6% rabbit serum (Sigma, St. Louis, MO) and 400 μg/ml kanamycin sulfate. Spirochetes initially were grown at 23°C and then were shifted to 37°C; they then were harvested at mid- to late logarithmic phase (4×10^7 to 8×10^7 cells/ml) by centrifugation at 8,000 × *g*, washed twice in CMRL (Gibco Invitrogen, Carlsbad, CA), and resuspended in RPMI 1640 (Gibco). Lysates were prepared by sonicating live organisms for 30 s using three separate 10-s bursts with a 550-Sonic Dismembrator (Fisher Scientific, Waltham, MA). Live spirochetes were heat killed by immersion in a 48°C water bath for 30 min. The efficacy of heat killing was 99.97% ± 0.02%, as confirmed by plating on Barbour-Stoenner-Kelly solid medium culture plates (102) in three independent experiments. Flow cytometry with live and heat-killed GFP-expressing *B. burgdorferi* was performed using the cell-permeant red fluorescent nucleic acid stain SYTO59 (Molecular Probes Invitrogen) as described previously (39, 40). The equivalence of live, lysed, and heat-killed samples was confirmed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and silver staining. *T. pallidum* subsp. *pallidum* (Nichols strain) was propagated by intratesticular inoculation of adult New Zealand White rabbits as described previously (59) in strict accordance with protocols approved by the UCHC Animal Care Committee. Treponemes were extracted from rabbit testes in CMRL, pelleted once by centrifugation at 14,000 × *g*, resuspended in RPMI 1640, and enumerated by dark-field microscopy using a Petroff-Hausser counting chamber (Hausser Scientific).

PBMC isolation and stimulation. PBMCs were isolated from EDTA-treated blood on Ficoll-Hypaque (Amersham Biosciences, Piscataway, NJ) gradients. The isolated cells were washed three times in Hanks' balanced salt solution, counted with a hemocytometer, and resuspended to a density of 1×10^6 cells/ml in RPMI 1640 containing 10% heat-inactivated fetal bovine serum. One-milliliter portions then were plated in 24-well culture plates either without an agonist, with Fluoresbrite carboxylated polystyrene microspheres (Polysciences, Inc., Warrington, PA), or with live, lysed, or, in some experiments, heat-killed spirochetes and incubated for designated time periods at 37°C in a humidified atmosphere consisting of 5% CO_2 and air. To inhibit phagocytosis, cell suspensions were preincubated for 1 h without or with 10 μg/ml cytochalasin D (Sigma) prior to the addition of spirochetes and an additional 8 h of incubation. PBMCs were stimulated with live *T. pallidum* in the presence or absence of 10% heat-inactivated (56°C for 30 min) human syphilitic serum pooled from five human immunodeficiency virus-seronegative donors (99). In some experiments, spirochetes remaining in culture supernatants at the conclusion of the 8-h incubation period were enumerated by dark-field microscopy. Lipopolysaccharide levels in culture media and reagents were determined by the *Limulus* assay (Cambrex, Walkersville, MD) to be <10 pg/ml.

qRT-PCR analysis of cytokine gene expression. PBMCs were incubated for 4 h without an agonist, with live or lysed spirochetes at a multiplicity of infection (MOI) of 10, or with 1 μm Fluoresbrite carboxylated polystyrene microspheres (Polysciences, Inc., Warrington, PA) at a bead-to-cell ratio of 10. At the conclusion of the incubation period, RNA was isolated from cell pellets using Trizol according to the instructions of the manufacturer (Invitrogen). Each RNA sample was evaluated qualitatively and quantitatively using a 2100 Bioanalyzer (Agilent, Santa Clara, CA). First-strand cDNA synthesis reactions were performed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) with 100-μl reaction mixtures containing 100 ng total RNA, Moloney murine leukemia virus reverse transcriptase, and random hexamers according to the manufacturer's instructions. PCR amplification of cDNAs (2.5-μl portions) was performed using 25-μl reaction mixtures containing 1× TaqMan Amplitaq Gold universal master mixture (Applied Biosystems) and commercially available primer-probe sets (Applied Biosystems) for tumor necrosis factor alpha (TNF-α) (Hs99999043_m1), IL-1β (HS00174097_m1), IL-6 (Hs00174131_m1), and gamma interferon (IFN-γ) (Hs00174143_m1). Internal standard curves for each gene were generated using equal volumes of reverse-transcribed quantitative PCR human reference total RNA (Clontech, Mountain View, CA) diluted to obtain concentrations ranging from 125 to 0.0125 ng/μl. Quantitative real-time PCR (qRT-PCR) gene expression assays for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were performed using identical aliquots of each cDNA as normalization controls. All amplification reactions were performed in triplicate; control reactions without reverse transcriptase also were performed to confirm the absence of contaminating DNA. Amplification reactions were performed with a 7900 HT Fast real-time PCR system (Applied Biosystems) using the following conditions: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Cycle threshold values for each gene were determined from the linear region of the corresponding amplification plots using software supplied by the manufacturer (Applied Biosystems). Expression levels of TNF-α, IL-6, IL-1β, and IFN-γ in each sample, normalized to the GAPDH level, were calculated based on the internal standard curves and were plotted as mean fold increases ± standard errors of the means relative to normalized transcript values for the unstimulated samples.

Flow cytometric assessment of FSC/SSC changes in PBMCs and internalization of Bb-GFP by monocytes. PBMCs were incubated with spirochetes for 8 h and then harvested by centrifugation for 5 min at 500 × *g*, washed once in fluorescence-activated cell sorting buffer (phosphate-buffered saline, 0.1% bovine serum albumin, 0.05% sodium azide), and incubated with 10 μg of purified human immunoglobulin G (Sigma) to block Fc receptors prior to staining with specific monoclonal antibody conjugates. Monocytes were identified by staining


(A)
TNF-α
IL-6
pro-IL-1β
IFN-γ
Fold Increase
Beads
Live
Lysed
*
(B)
Unstimulated
0.1%
Live Bb
Lysed Bb
Control
Osp A
Osp C
75kD
50kD
37kD
25kD
15kD
10kD
1:1
10:1
100:1
Live
Lysed
6.0%
51.2%
97.0%
0.3%
1.3%
9.3%
GFP B. burgdorferi
CD14

with anti-CD14 conjugated with phycoerythrin (eBioscience, San Diego, CA). DCs were identified as lineage$^-$/HLA-DR$^+$ cells after staining with a fluorescein isothiocyanate-labeled lineage cocktail (BD Biosciences, San Jose, CA) and anti-HLA-DR conjugated with peridinin chlorophyll protein (BD Biosciences). T cells were identified using anti-CD3-fluorescein isothiocyanate (BD Biosciences). To assess phagocytosis of *B. burgdorferi*, lymphocytes and debris were gated out of the total cell population on the basis of forward scatter (FSC)/side scatter (SSC) properties, and the GFP fluorescence (FL1 channel) of the remaining CD14$^+$ cells was determined. Flow cytometry was performed with a FACSCalibur dual-laser flow cytometer (BD Biosciences); 250,000 events were collected and analyzed using WinMDI v2.8 software (http://facs.scripps.edu/software.html).

TUNEL assays. PBMCs were incubated for 4 h with live, lysed, or heat-killed *B. burgdorferi* at MOIs ranging from 1 to 100, with microspheres at bead-to-cell ratios of 10, or with 1 μM staurosporine (Sigma). The cells then were washed, resuspended in 1% paraformaldehyde, placed on ice for 30 min, washed twice in phosphate-buffered saline, and resuspended in 70% ethanol. Terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) reactions and staining with anti-bromodeoxyuridine triphosphate (BrdUTP) were performed using an Apo-BrdU-Red in situ DNA fragmentation assay kit according to the instructions of the manufacturer (BioVision, Mountain View, CA). For flow cytometry, 50,000 events were acquired using the double-discrimination mode for FL3 to facilitate the gating out of events containing more than 2 N of DNA (i.e., aggregated cells). Bromodeoxyuridine positivity in monocytes and lymphocytes was assessed based on FSC/SSC properties after gating out of debris. For each cell type, the percentage of BrdUTP-positive cells was defined as the fraction of cells with increased fluorescence compared to the corresponding population of uninfected controls.

DAPI staining. PBMCs were incubated for 6 h with Bb-GFP at an MOI of 10 or 100 or with 1 μM staurosporine and subsequently harvested and fixed in 1% paraformaldehyde for 20 min. In some cases, 50 nM LysoTracker Red was added to the cultures. DNA staining was performed by adding 5 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen) for 10 min. After two washes in phosphate-buffered saline, cells were mounted on slides and examined by phase-contrast microscopy with a Nikon Eclipse E600 microscope or an Olympus Bx60 mercury arc lamp fluorescence microscope, both with oil immersion (magnification, ×1,000). Image overlays were obtained with ImageJ v1.36b (http://rsb.info.nih.gov/ij/).

Measurement of cytokine levels. Levels of TNF-α, IL-1β, and IFN-γ in 8-h culture supernatants were measured using a human inflammatory cytokine bead array kit according to the protocol of the manufacturer (BD Biosciences). Intracellular staining for IFN-γ production by NK cells was performed as described previously (88).

Statistical analysis. Statistical analysis was performed using standard spreadsheet software (Excel; Microsoft, Inc., Redmond, WA) and GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Fold increases in cytokine transcript levels were compared using a single-tailed paired Student *t* test. All other comparisons were carried out using two-tailed Student's *t* tests. For each experiment, both the standard deviation and the standard error of the mean were calculated. *P* values of ≤0.05 were considered significant.

RESULTS

Phagocytosis of live *B. burgdorferi* induces enhanced transcription of proinflammatory cytokine genes. Previously, we used freshly isolated human PBMCs to investigate the interactions between innate immune cells and LD spirochetes (88). We found that in this ex vivo system, live *B. burgdorferi* elicited greater secretion of proinflammatory cytokines from monocytes than the corresponding lysates elicited and that this enhanced response was phagocytosis dependent. Because TLR-derived signals can promote cytokine secretion by post-transcriptional as well as transcriptional mechanisms (89, 113), at the outset we sought evidence that phagocytosis-triggered signals act at the transcriptional level. To this end, we used qRT-PCR to measure cytokine gene transcripts in PBMCs incubated with no bacteria or with live or lysed *B. burgdorferi* at a relatively low MOI (MOI, 10) or with polystyrene beads. Figure 1A shows GAPDH-normalized values relative to the values for suspensions incubated without bacteria. Live *B. burgdorferi* induced significantly greater amounts of transcripts for TNF-α, IL-6, and pro-IL-1β than lysates induced, while polystyrene beads had no effect. Whereas the difference between the pro-IL-1β transcript levels in PBMCs stimulated with live or lysed *B. burgdorferi* was approximately 2-fold, there was a nearly 40-fold difference in cytokine secretion induced by these agonists at the same MOI (see Fig. 7). Thus, in addition to inducing enhanced NF-κB-dependent cytokine gene transcription, phagocytosed spirochetes deliver a potent signal for activation of caspase-1, the cytosolic cysteine proteinase required for secretion of biologically active IL-1β (80, 84). Another noteworthy discovery made with our ex vivo model is that internalization of live *B. burgdorferi* by DCs induced the production of IFN-γ from innate lymphocytes, principally NK cells (88). In accord with this result, we found that transcription of IFN-γ was strongly induced exclusively by live *B. burgdorferi* (Fig. 1A). We also determined by flow cytometry that monocytes internalized much greater amounts of live spirochetes than lysed spirochetes (Fig. 1B); in these experiments, it is possible that diffusion of GFP from lysates resulted in an underestimate of the degree of internalization. Moreover, SDS-PAGE confirmed that live and lysed samples were equivalent with respect to protein content (Fig. 1B, inset). The latter results support the contention that lipoproteins in lysed *B. burgdorferi* interact predominantly with pattern recognition receptors on the surface of phagocytic cells and that enhanced stimulation by live *B. burgdorferi* reflects signaling events induced by internalization of the microbe.

Phagocytosis of live *B. burgdorferi*, but not phagocytosis of lysed *B. burgdorferi*, induces a programmed cell death response in monocytes. During the course of our experiments, we made an unexpected observation: not only did live *B. burgdorferi* induce much greater activation of monocytes than did lysed spirochetes, it also resulted in what appeared to be a reproducible and dose-dependent diminution in the number of monocytes remaining at the conclusion of an 8-h incubation

FIG. 1. Phagocytosis of live *B. burgdorferi* results in enhanced transcription of proinflammatory cytokine genes. (A) PBMCs from five healthy volunteers were incubated for 4 h with live or lysed *B. burgdorferi* at an MOI of 10 or with polystyrene microspheres at a bead-to-cell ratio of 10. RNAs then were isolated for measurement of cytokine gene transcripts by qRT-PCR. The *y* axes show the fold increases in GAPDH-normalized values for TNF-α, pro-IL-1β, IL-6, and IFN-γ relative to suspensions incubated without bacteria; the bars indicate the means, and the error bars indicate the standard errors of the means. An asterisk indicates that the *P* value is <0.05 (for comparisons of live *B. burgdorferi* and lysates). (B) Monocytes in PBMCs incubated for 8 h with live or lysed Bb-GFP at the indicated MOIs were analyzed for GFP fluorescence by flow cytometry. Note that all monocytes, including those incubated with live spirochetes at an MOI of 100, remained strongly CD14$^+$. The percentage of GFP-positive cells is indicated in each cytogram. The cytograms are representative of three independent experiments. SDS-PAGE (inset) confirmed that the incubation mixtures contained equivalent amounts of live or lysed *B. burgdorferi*.

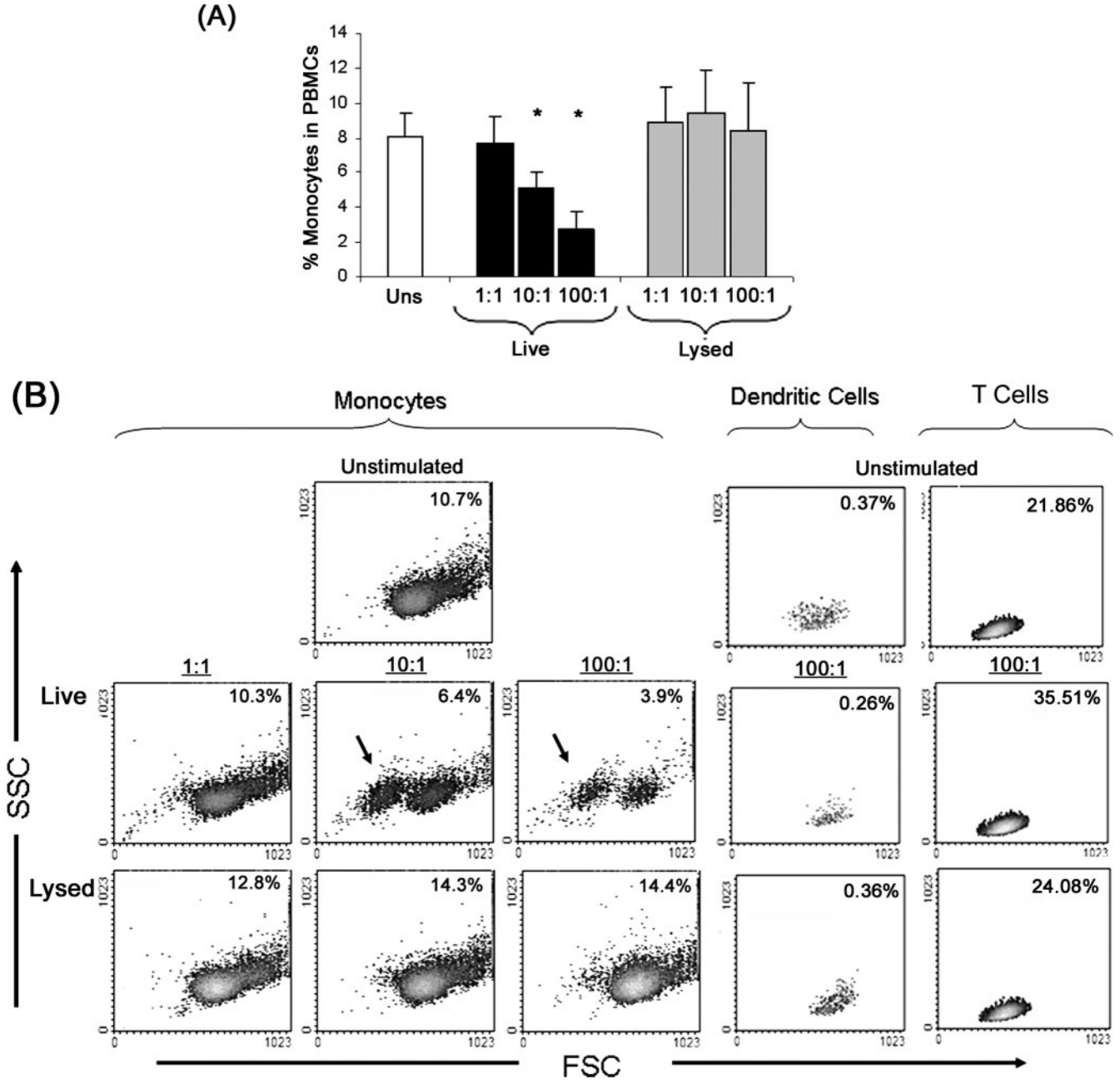


FIG. 2. Live *B. burgdorferi*, but not lysed *B. burgdorferi*, induces monocyte loss and shrinkage in PBMCs. (A) Enumeration of monocytes in PBMCs following 8 h of incubation with graded doses of live or lysed *B. burgdorferi*. The bars indicate the averages and the error bars indicate the standard errors of the means of six independent experiments. An asterisk indicates a value that is statistically significantly different ($P \leq 0.05$) than the value for unstimulated (Uns) cultures. (B) FSC/SSC profiles of monocytes, DCs, and T cells from PBMCs incubated with live or lysed *B. burgdorferi* at graded MOIs. The percentages of each immune cell type in the PBMC suspensions are indicated in the density plots. Monocytes with altered SSC/FSC properties are indicated by arrows. The cytograms for the monocytes are representative of six independent experiments, while those for DCs and T cells are representative of three independent experiments. The cytograms were obtained using PBMCs from the same individual.

period (Fig. 2A). As surface expression of CD14 on monocytes has been reported to diminish following incubation with lipopolysaccharide and whole bacteria (28, 73, 77), we considered the possibility that the ostensible decrease in monocyte numbers was due to down-regulation of this surface marker. While we did, in fact, detect mean fluorescence intensity decreases of approximately 20 and 40% in monocytes incubated with live organisms at MOIs of 10 and 100, respectively (data not shown), these modest changes did not impede our ability to identify monocytes within the PBMCs, even at the highest MOI employed (Fig. 1B). Two additional findings confirmed that an actual decrease in monocyte numbers occurred in the

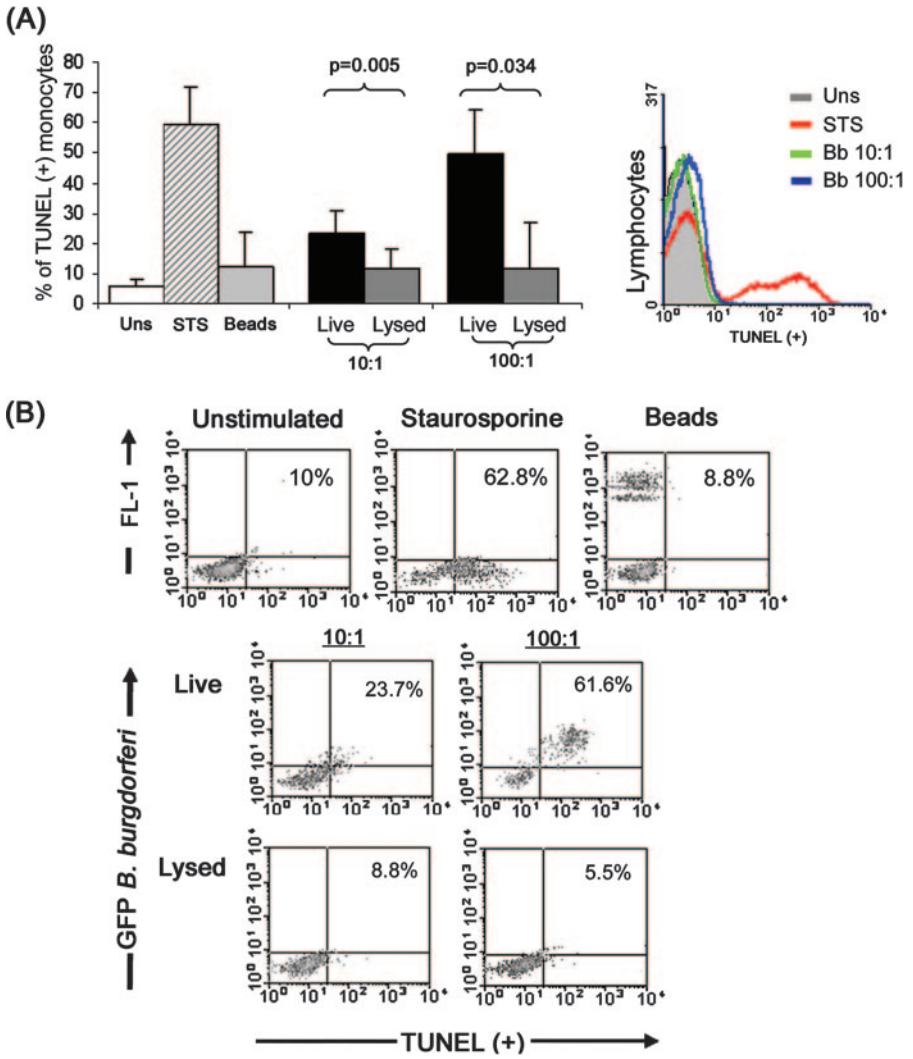


FIG. 3. Live *B. burgdorferi* induces DNA fragmentation predominantly in monocytes containing spirochetes. (A) PBMCs were incubated for 4 h with 1 μM staurosporine (STS), with fluorescent carboxylate beads (bead-to-cell ratio, 10), or with live or lysed *B. burgdorferi* (Bb) (MOI, 10 or 100) and then assayed for DNA fragmentation by determination of TUNEL activity. Monocytes and lymphocytes were identified via their distinctive FSC/SSC properties. The monocyte graphs show the means and standard errors of the means for four independent experiments; the lymphocyte histogram is representative of the histograms obtained in four experiments. (B) PBMCs incubated with live or lysed Bb-GFP were assayed as described above and analyzed by flow cytometry. Monocytes stimulated with staurosporine became TUNEL positive but remained GFP negative; cells incubated with inert fluorescent beads became GFP positive without evidence of DNA disruption. The percentage of TUNEL-positive cells is indicated in each cytogram. The density plots are representative of four independent experiments.

incubation mixtures containing live organisms. First, a reduction in the number of cells with monocyte-like FSC/SSC properties was readily apparent upon inspection of the FSC/SSC profiles of the entire PBMC population (data not shown). Second, in PBMCs incubated with live spirochetes, but not in PBMCs incubated with lysates, we observed the de novo appearance of a population of $CD14^+$ cells which were clearly smaller than either the monocytes in the unstimulated PBMC suspensions or the "normal-sized" monocytes remaining at the conclusion of the incubation period (Fig. 2B). It was noteworthy that we did not observe parallel decreases in either the size or numbers of T cells and DCs in PBMCs containing viable organisms (Fig. 2B).

The flow cytometric changes observed in monocytes incubated with live *B. burgdorferi* are characteristic of apoptosis or programmed cell death (75, 120). We next performed TUNEL assays to determine if *B. burgdorferi* induced DNA fragmentation in monocytes, another hallmark of apoptosis (45, 120). As shown in Fig. 3A, we found statistically significant dose-dependent incorporation of BrdUTP in monocytes stimulated for 4 h with live *B. burgdorferi*, but not in monocytes stimulated for 4 h with lysed *B. burgdorferi*, at MOIs of 10 and 100 (Fig. 3A). In contrast, TUNEL positivity was not observed in lymphocytes incubated with viable spirochetes at any MOI (Fig. 3A), consistent with the absence of flow cytometric changes observed in this population (Fig. 2B). Whereas induction of cell death by *B. burgdorferi* was restricted to monocytes, the protein kinase C inhibitor staurosporine, a well-characterized proapoptotic compound (43, 53), caused DNA disruption in both monocytes and lymphocytes (Fig. 3A). We next used Bb-GFP to deter-

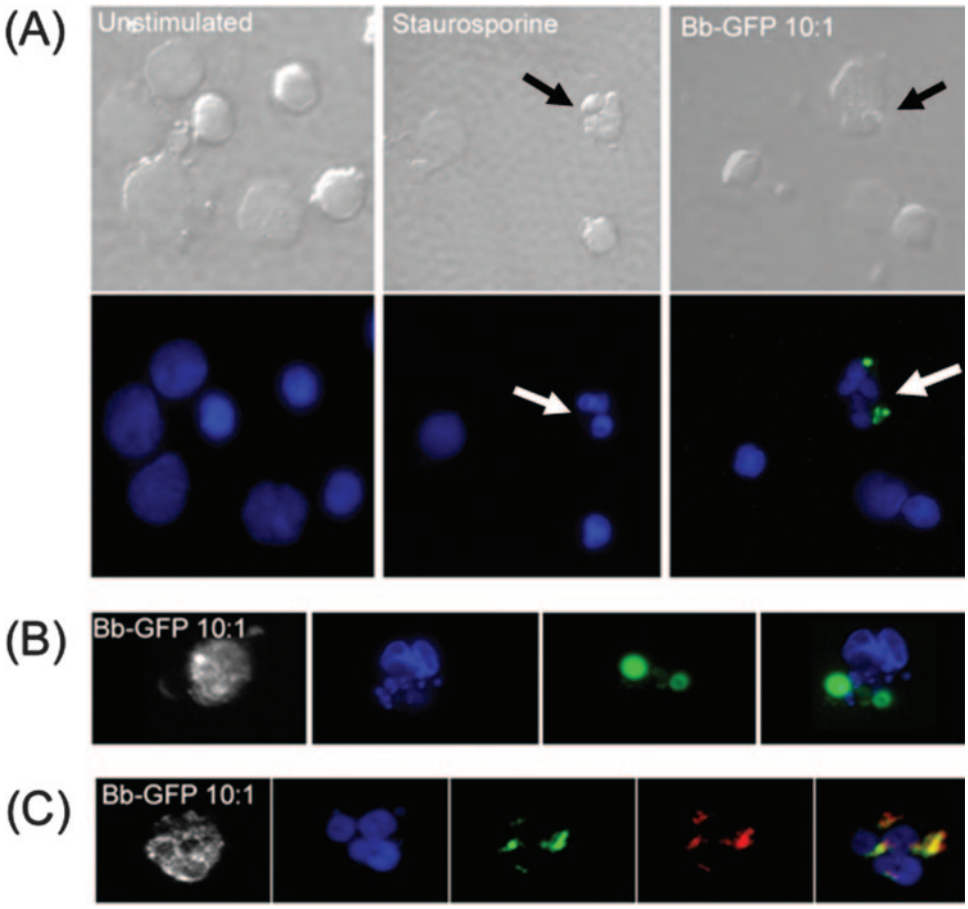


FIG. 4. Internalization of live spirochetes induces morphological changes consistent with programmed cell death. PBMCs incubated for 6 h with Bb-GFP at an MOI of 10 or with staurosporine (1 μM) were stained with DAPI and examined for microscopic evidence of programmed cell death. (A) Phase-contrast microscopy (top row) revealed blebbing (arrows) in cells stimulated with staurosporine or *B. burgdorferi*. With fluorescence microscopy (bottom row), the same cells exhibited nuclear fragmentation and condensation as determined by DAPI staining (arrows). GFP can be seen in the micrograph showing an apoptotic cell incubated with Bb-GFP. (B) Localization of GFP in cells demonstrating nuclear disruption. (C) Costaining of cells with LysoTracker Red confirmed that the GFP was confined to lysosomal compartments.

mine if there was a correlation between the internalization of spirochetes and DNA fragmentation. The cytograms in Fig. 3B indicate that DNA fragmentation occurred predominantly in cells that had ingested spirochetes. Notably, cells that internalized inert fluorescent beads did not become TUNEL positive, indicating that chromatin cleavage is not a result of phagocytosis per se.

Other canonical features of apoptosis were sought by light microscopy. Using phase-contrast microscopy, blebs were readily observed on the surfaces of cells stimulated with live *B. burgdorferi* or staurosporine (Fig. 4A). Fluorescence microscopy of PBMCs stained with the nucleic acid dye DAPI revealed nuclear condensation and fragmentation in cells that were incubated with live Bb-GFP (Fig. 4A). The correlation between cells displaying nuclear disruption and internalization of Bb-GFP was clearly evident (Fig. 4A and B). Also noteworthy was the finding that the internalized spirochetes were completely degraded to amorphous fluorescent material which, based on costaining with LysoTracker Red, appeared to be confined to phagocytic vesicles (Fig. 4C). Previously, we showed that preincubation of PBMCs with cytochalasin D (10 μg/ml) blocked phagocytosis of spirochetes and markedly diminished proinflammatory cytokine production (88). As shown in Fig. 5, blockage of phagocytosis with cytochalasin D virtually eliminated the loss of monocytes in PBMCs incubated with live *B. burgdorferi*, thereby providing further evidence for a causal relationship between these two phenomena.

Phagocytosis of heat-killed *B. burgdorferi* induces programmed cell death but diminished cytokine output. We next asked whether internalized spirochetes must be viable in order to induce cell death and/or cell activation. Unlike the outer membranes of gram-negative bacteria, the outer membranes of *B. burgdorferi* are fragile and easily damaged during physical manipulations (22, 31). To perform these experiments, therefore, it was first necessary to develop a method for generating nonviable but structurally intact organisms that would retain GFP expression and be phagocytosed at levels comparable to those of live Bb-GFP. The protocol that we developed (see Materials and Methods) killed more than 99.9% of the organisms (data not shown), yet it preserved gross spirochetal morphology (Fig. 6A) and polypeptide composition as determined by SDS-PAGE (data not shown). Furthermore, as determined

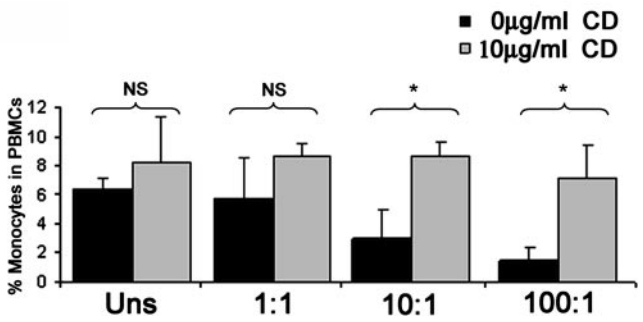


FIG. 5. Inhibition of phagocytosis prevents loss of monocytes. Cell suspensions were preincubated for 1 h without or with 10 μg/ml cytochalasin D (CD) (Sigma) prior to the addition of spirochetes. The numbers of monocytes remaining after a subsequent 8 h of incubation were determined by flow cytometry. The bars indicate the means and the error bars indicate the standard errors of the means for three independent experiments. An asterisk indicates that there was a statistically significant difference. Uns, unstimulated; NS, not significant.

by both light microscopy and flow cytometry, heat-killed spirochetes retained a high level of fluorescence (Fig. 6A). Subsequent examination by flow cytometry (Fig. 6B and 6C) and light microscopy (not shown) revealed that heat-killed *B. burgdorferi* was internalized by monocytes as well as live spirochetes, strongly suggesting that the heat-killing procedure did not damage spirochetal surface structures critical for monocyte binding and uptake. Heat-killed *B. burgdorferi* also induced FSC/SSC changes in monocytes indicative of cell shrinkage (Fig. 6C), dose-dependent decreases in monocyte numbers (Fig. 6D), and DNA fragmentation as determined by the TUNEL assay (Fig. 6E) comparable to the changes induced by viable *B. burgdorferi*. As it did with live *B. burgdorferi*, TUNEL assay positivity showed a strong correlation with internalization of heat-killed *B. burgdorferi* (Fig. 6E). In contrast, live and heat-killed organisms demonstrated substantially different capacities for activation of monocytes and DCs. Heat-killed *B. burgdorferi* induced significantly less TNF-α and IL-1β secretion than live *B. burgdorferi* induced (Fig. 7), while IFN-γ production in response to heat-killed *B. burgdorferi* was not detectable by an assay of culture supernatants (data not shown). Use of the more sensitive intracellular cytokine staining technique (88) revealed that heat-killed *B. burgdorferi* did induce production of some IFN-γ by NK cells, although the amount was clearly less than the amount induced by live Bb-GFP (Fig. 7).

Ospsonized *T. pallidum* does not induce a death response in monocytes. *T. pallidum* is poorly internalized in the absence of opsonizing antibody (72, 95). Recently, we showed that human secondary syphilitic serum promoted opsonophagocytosis of *T. pallidum* by monocytes and that uptake of opsonized treponemes induced levels of monocyte activation and inflammatory cytokine production comparable to the levels elicited by live *B. burgdorferi* (88). Thus, it was of interest to determine if opsonized *T. pallidum* elicited a comparable programmed cell death response. Unexpectedly, opsonized *T. pallidum* induced neither a significant loss of monocytes from PBMCs (Fig. 8A) nor changes in the FSC/SSC profiles (Fig. 8B). To rule out the possibility that the dichotomy in the death responses induced by the two spirochetes was due to less proficient uptake of *T. pallidum*, we assessed internalization efficiencies by enumerating residual spirochetes in culture supernatants following 8-h incubation periods. As shown in Fig. 8C, PBMCs ingested similar percentages of *B. burgdorferi* and opsonized *T. pallidum* at equivalent MOIs. The dramatic effect of human syphilitic serum on internalization of *T. pallidum* was evident from the finding that the levels of unopsonized *T. pallidum* recovered were very similar to the input levels (Fig. 8C).

DISCUSSION

Investigations with individual pathogen-associated molecular patterns (PAMPs) and transfected cell lines have promoted the viewpoint that recognition of extracellular bacteria occurs mainly via the interaction of cell wall constituents with TLRs on the surface of innate immune cells (3, 25). Recent studies with mammalian systems have revealed the existence of a cytosolic pathogen surveillance network comprised of a superfamily of Nod-like receptors (80, 84). Based on experiments utilizing vacuole-restricted mutants of intracellular pathogens, investigators often have surmised that confinement of extracellular bacteria to phagosomal compartments segregates them from cytosolic sensors (48, 81, 85). Work in our laboratory using human PBMCs incubated with live spirochetes has indicated that both of the above-described ideas regarding innate immune recognition of extracellular bacteria are overly simplistic and that cytosolic signals induced by phagocytosed *B. burgdorferi* induce programmed cell death as well as cellular activation.

Since the first report of macrophage apoptosis following infection with *Shigella flexneri* in 1992 (127), the ability of parasites to alter the balance between pro- and antiapoptotic signals in phagocytes has generated considerable interest (35, 47). Using microarrays, Kobayashi et al. (70) documented the rapid induction by *Borrelia hermsii* of a proapoptotic genetic program in human neutrophils. Despite the many studies of *B. burgdorferi*-phagocyte interactions that have appeared over the years, our report and a recent communication by Glickstein and Coburn (52) are the only papers to describe the induction of cell death by LD spirochetes in mononuclear phagocytes. In their seminal study of TLR2-mediated cell activation, Aliprantis et al. (9) observed, using transfected HEK293 cells, that bacterial lipoproteins/lipopeptides have proapoptotic as well as cytokine-inducing properties. Aliprantis et al. (10) and other workers (62) subsequently reported that the TLR2-mediated inflammatory and cell death responses to bacterial lipoproteins bifurcate at the level of myeloid differentiation factor 88, with the former proceeding through NF-κB and the latter engaging the extrinsic apoptotic pathway components Fas-associated death domain protein and caspase 8. In contrast, studies with murine macrophages have failed to confirm that TLR2 signaling is intrinsically apoptogenic (55–57, 98). Similarly, in our PBMC system, *B. burgdorferi* lysates, which are highly enriched for lipoproteins and potent TLR2 agonists (122), were unable to induce apoptosis in normal human monocytes, even at extremely high MOIs. The inability of phagocytosed *T. pallidum* to induce apoptosis also argues against the notion that lipoproteins are intrinsically proapoptotic for human mononuclear phagocytes.

Stuart and Ezekowitz (114) coined the term "phagocytic

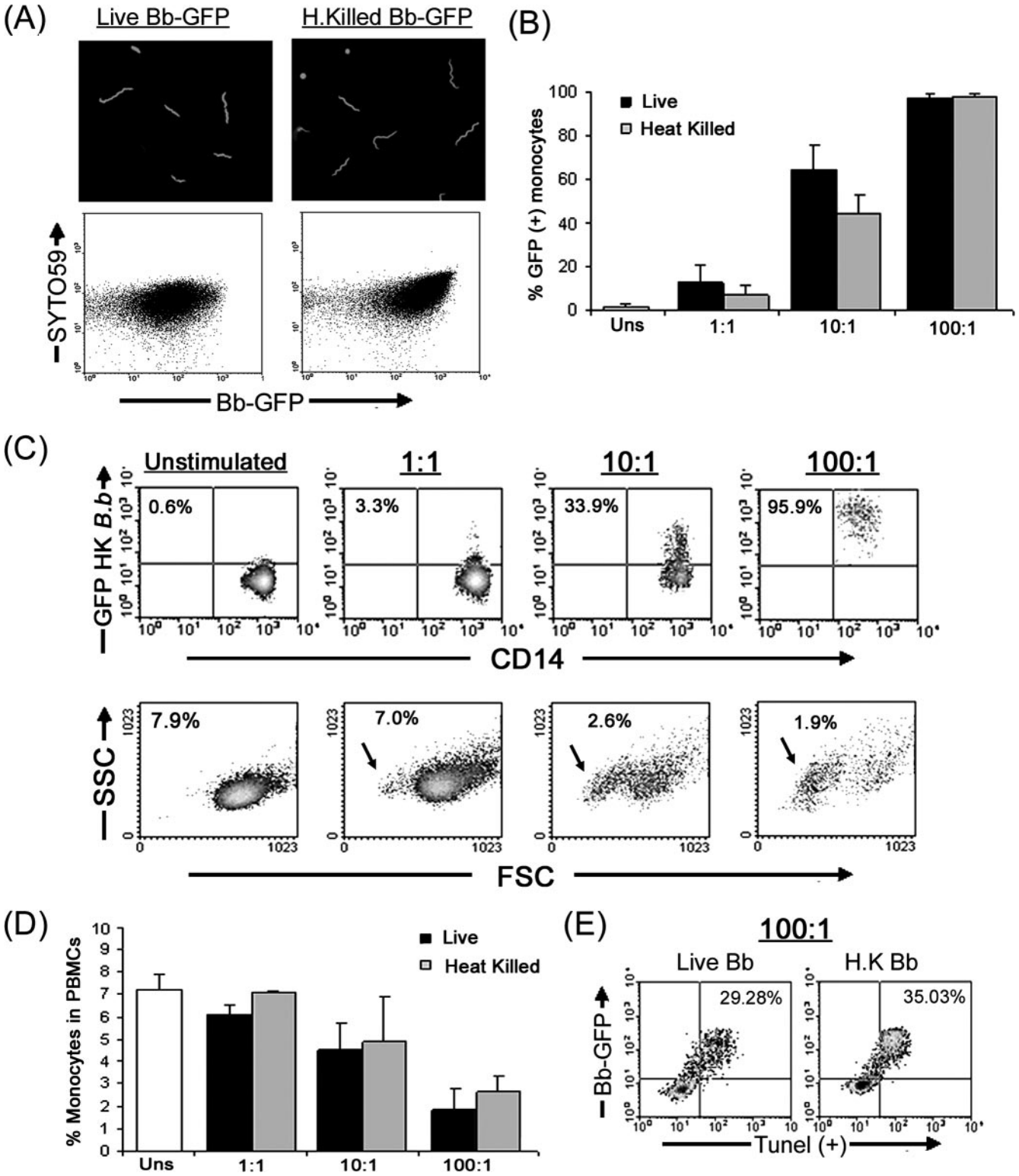


FIG. 6. Heat-killed *B. burgdorferi* and viable *B. burgdorferi* induce comparable levels of apoptosis following internalization by monocytes. (A) Fluorescence microscopy and flow cytometry of live and heat-killed (H.Killed) Bb-GFP. The density plots from one of two separate analyses are shown. Staining with the cell-permeant nucleic acid dye SYTO59 was used to confirm that GFP fluorescence was associated with organisms. (B) Internalization of live and heat-killed Bb-GFP by monocytes as determined by flow cytometry. The bars indicate the means and the error bars indicate the standard deviations for three independent experiments. None of the differences was statistically significant. Uns, unstimulated. (C) Internalization of heat-killed *B. burgdorferi* (HK *B.b*) by monocytes induces changes in FSC/SSC properties consistent with cell shrinkage. The percentages in the top panels indicate the percentages of GFP-positive monocytes, while the percentages in the lower panel indicate the proportions of monocytes remaining in PBMC suspensions. The arrows indicate monocytes with altered FSC/SSC properties. The cytograms are

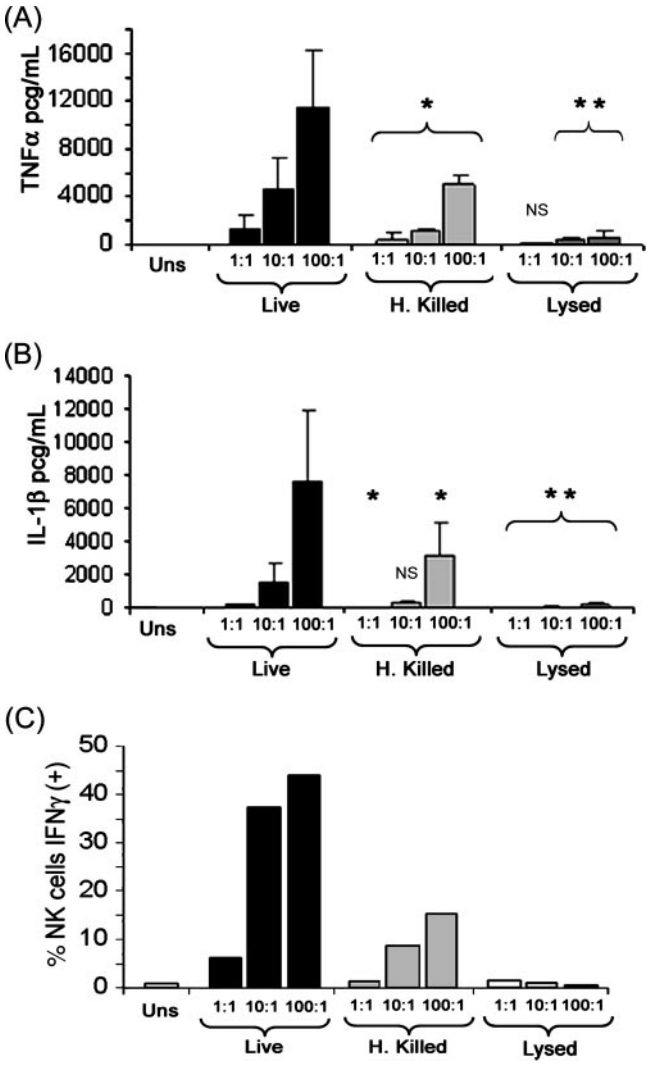


FIG. 7. Heat-killed *B. burgdorferi* induces a significantly diminished inflammatory cytokine response compared to live *B. burgdorferi*. TNF-α (A) and IL-1β (B) levels were measured in culture supernatants following 8 h of incubation of PBMCs with live, heat-killed (H. Killed), or lysed spirochetes at the indicated MOIs. One asterisk indicates a *P* value of ≤0.05 for a comparison between live *B. burgdorferi* and heat-killed *B. burgdorferi* at equivalent MOIs; two asterisks indicate a *P* value of ≤0.05 for a comparison between heat-killed *B. burgdorferi* and lysed *B. burgdorferi* at equivalent MOIs. The bars indicate the means and the error bars indicate the standard deviations for four independent experiments. (C) IFN-γ production by NK cells as measured by intracellular cytokine staining in two independent experiments. Uns, unstimulated; NS, not significant.

synapse" to denote the array of surface receptors that are believed to act cooperatively during phagocytosis to bind a bacterium, trigger internalization, and initiate signaling cascades that determine the fates of both the bacterium and the engulfing cell. Our finding that cytochalasin D dramatically diminished cellular responsiveness (88) and apoptosis indicates that the enhanced recognition of live *B. burgdorferi* associated with phagocytosis cannot simply be the result of the combinatorial input from multiple receptors on the nascent phagosome but rather must reflect signaling events that occur after the ingestion of the bacterium. Phagosomes undergo extensive remodeling of lipid and protein components as they attain functional maturity (21, 37, 107, 114, 118). As maturation proceeds, the phagosome likely gains the ability to recognize microbial patterns and/or becomes an increasingly efficient platform for signal generation and assembly of novel signaling complexes. When we used LysoTracker Red for intracellular localization of spirochetes taken up by monocytes in PBMCs, we saw mainly degraded organisms within phagolysosomes. Using a combination of light and transmission electron microscopy, Montgomery and Malawista (87) found that spirochetes were swiftly degraded following uptake by murine macrophages. The liberation of *B. burgdorferi* constituents as fragments that can be accommodated within the binding sites of their cognate pattern recognition receptors is likely critical for the phagocytosed spirochete's potency as an initiator of innate cellular responses.

The ex vivo system utilized here illustrates the complexity of signaling events that underlie recognition of live bacteria, as well as the difficulties in relating these pathways to individual microbial patterns. We found that phagocytosis of spirochetes enhances or generates de novo three signaling cascades. The first signaling cascade leads to the production of NF-κB-dependent cytokines and, presumably, is TLR mediated. Several years ago, Aderem and colleagues (93, 119) showed that TLR2 and its cooperative pairing partners TLR1 and TLR6 are recruited to the phagosome in transfected RAW 264.7 cells following ingestion of zymosan particles; recruitment of TLR2 to phagosomes in untransfected RAW cells subsequently was demonstrated by Nilsen et al. (92). The method that we devised for gently heat killing *B. burgdorferi* enabled us to deliver to phagosomes a cargo of spirochetal proteins, including lipoproteins, equivalent to that introduced with live organisms. Despite being efficiently phagocytosed, heat-killed *B. burgdorferi* elicited significantly diminished production of TNF-α, IL-6, and IL-1β by monocytes compared to viable spirochetes. The much diminished production of IFN-γ by bystander NK cells indicated that the presence of heat-killed *B. burgdorferi* also resulted in diminished activation of DCs (88). Our results, therefore, point to the existence of one or more nonlipoprotein PAMPs which signal from within the vacuole. A similar deduction was made for the murine system by Coleman and Benach (29), who found that peritoneal macrophages from TLR2-deficient mice strongly upregulated the expression of the urokinase receptor in response to live spirochetes, whereas the response to lysed organisms was abrogated in mice lacking

representative of three independent experiments. (D) Live and heat-killed *B. burgdorferi* induce comparable losses of monocytes from PBMCs. The bars indicate the means and the error bars indicate the standard deviations for three independent experiments. (E) Correlation of internalization with induction of DNA fragmentation, as determined by the TUNEL assay. The percentage of TUNEL-positive monocytes is indicated in each cytogram. Representative results from two independent experiments are shown. Live Bb, live *B. burgdorferi*; H.K Bb, heat-killed *B. burgdorferi*.

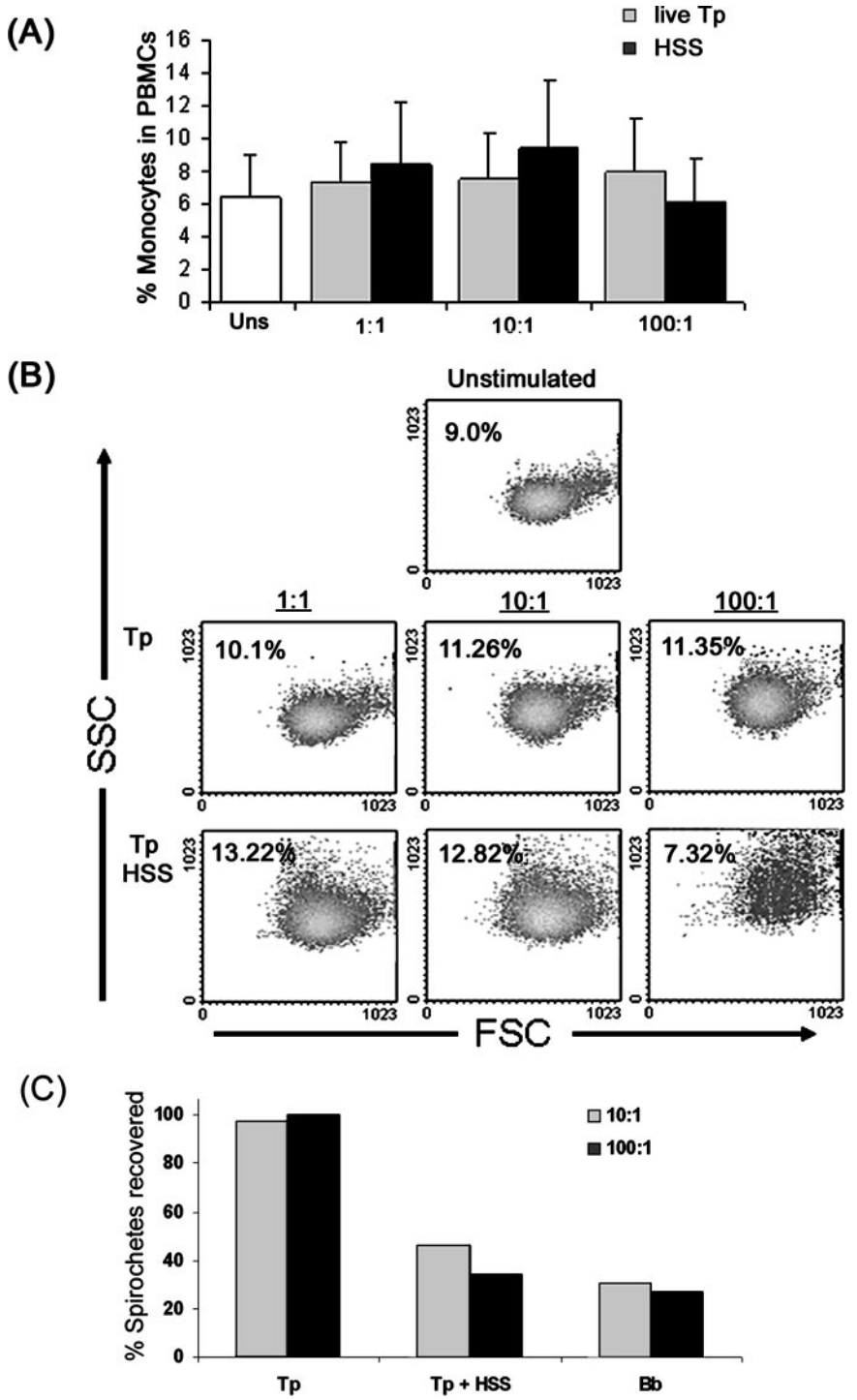


FIG. 8. Opsonophagocytosis of *T. pallidum* does not induce programmed cell death in monocytes. PBMCs were incubated for 8 h with live *T. pallidum* at an MOI of 1, 10, or 100 in the presence or absence of 10% heat-inactivated human syphilitic serum. Changes in monocyte (A) numbers and (B) FSC/SSC properties were assessed by flow cytometry after an 8-h incubation period. The percentages of monocytes remaining in PBMC suspensions are indicated in the cytograms in panel B. In panel A the bars indicate the means and the error bars indicate the standard errors of the means from five independent experiments. No statistically significant differences were observed when opsonized *T. pallidum* was compared with unstimulated controls. (C) Percent recovery of *T. pallidum* (in the absence or presence of human syphilitic serum) and *B. burgdorferi* following 8 h of incubation with PBMCs. The values for *T. pallidum* and *B. burgdorferi* are the means of two and three experiments, respectively. Tp, *T. pallidum*; Bb, *B. burgdorferi*; Uns, unstimulated; HSS, human syphilitic serum.

TLR2. Flagellin is unlikely to be the unidentified PAMP because live and heat-killed samples contain identical amounts of this protein. Moreover, TLR5, the receptor for flagellin, is well expressed in monocytes but is not known to be phagosome associated (25, 63, 112).

Since phagosomal recruitment of TLR2, TLR1, and TLR6 was reported (93, 119), the ligands for TLRs that signal exclusively from within vesicular compartments have been identified as DNA (TLR9), double-stranded RNA (TLR3), and single-stranded RNA (TLR7/TLR8) (3, 25, 67). Human monocytes do not express TLR3 or TLR9, but they do express TLR7 and TLR8 and respond well to TLR7/TLR8 agonists (15, 54, 63). Cooperation between TLRs from different cellular compartments has been well documented (116). We, therefore, propose TLR7/TLR8 and *B. burgdorferi* RNA as candidate TLR-ligand pairs that function cooperatively with TLR2/TLR1 for optimal TLR stimulation by phagocytosed *B. burgdorferi*. While TLR7 and TLR8 have generally been conceived of as mediating responses to viral RNAs, we know of no theoretical reason why they could not recognize RNAs derived from bacteria. Adding to the plausibility of this notion are findings that liposome transfection of monocyte-derived DCs with bacterial, but not eukaryotic, RNA strongly induced secretion of IL-12 (71). Because endosomal TLRs are potent activators of type I IFN and related pathways (3, 76), this proposed scenario for cooperative TLR signaling by live *B. burgdorferi* could explain why type I IFNs and type I IFN-responsive genes were strongly upregulated in joint tissues of *B. burgdorferi*-infected mice (32). Recently, Kanneganti and colleagues (66) reported that bacterial RNA can activate caspase-1 through the cryopyrin/NALP3 inflammasome (80). RNA derived from phagocytosed *B. burgdorferi* could, therefore, serve a dual signaling function by contributing to the TLR-mediated priming that leads to the synthesis of pro-IL-1β and providing a TLR-independent stimulus for the activation of caspase-1 and secretion of IL-1β, the second signaling pathway enhanced by phagocytosis of live spirochetes. How *B. burgdorferi* RNA could be delivered to the cytosol for binding to a Nod-like receptor remains an open question.

Apoptosis, the third signaling cascade induced by phagocytosed spirochetes, traditionally has been described as immunologically quiescent (45, 79). In the context of monocyte-bacterium interactions, however, the distinction between apoptosis and immunity can be obscure because the two processes are often closely linked temporally and physiologically (33, 35). *Salmonella*- and *Shigella*-induced cell death in murine macrophages, which has an absolute requirement for caspase-1, is the prototype for the convergence of cell death and proinflammatory signal transduction pathways following the internalization of a bacterial pathogen (61, 65, 86). The high levels of IL-1β elicited in PBMCs incubated with live spirochetes might suggest that the similarly rapid demise of monocytes following the uptake of *B. burgdorferi* also was caspase-1 dependent. Three lines of evidence argue that the proinflammatory and apoptotic responses to *B. burgdorferi* are mediated by distinct signaling pathways. First, the atypical, rapid programmed cell death caused by infection with *Salmonella* and *Shigella* (61) results from the formation of a unique inflammasome containing Ipaf, whereas the NALP3/cryopyrin inflammasome, which is believed to function as a general sensor for cytosolic "danger signals," does not, as a matter of course, cause cellular demise (80, 84, 115). Second, we found that apoptosis was essentially unaffected when PBMCs were incubated with heat-killed *B. burgdorferi*, while the output of cytokines was markedly diminished. Lastly, while *T. pallidum* is much more resistant to phagocytosis than *B. burgdorferi* because of its poorly antigenic outer membrane, it is internalized well and is strongly proinflammatory when it is incubated with PBMCs in the presence of the opsonic antibodies in human syphilitic serum (88). In contrast to *B. burgdorferi*, phagocytosis of *T. pallidum* did not result in discernible monocyte apoptosis even when large numbers were ingested.

Most apoptotic responses are initiated either by ligand binding to a death receptor on the cell surface (the extrinsic pathway) or by intracellular stressors which result in the loss of integrity of the outer mitochondrial membrane and the release of apoptogenic molecules, such as cytochrome *c*, into the cytosol (the intrinsic pathway) (1, 97). In recent years, a number of investigators have shown that phagocytosis of bacteria, including nonpathogens, can trigger intrinsic apoptotic pathways (4, 35, 57, 68, 70, 83, 91, 94, 117, 121). These studies collectively provide an attractive working hypothesis for the observations reported here. The rapid kinetics of cellular demise during incubation with *B. burgdorferi*, coupled with the finding that apoptotic changes occurred predominantly in monocytes that had ingested LD spirochetes, are consistent with activation of the intrinsic pathway as the primary road to cell death. It is important to note, however, that this conjecture neither precludes a significant contribution from the extrinsic pathway, which can be stimulated by phagocytosis-mediated cell activation (12, 20), nor requires that spirochete constituents serve directly as cytosolic death signals. Both reactive nitrogen and oxygen intermediates, which are generated in large amounts during phagocytosis as part of the monocyte's antimicrobial arsenal, have intracellular signaling functions (17, 41, 44) and have been associated with pathogen-induced phagocyte apoptosis (6, 8, 12, 16, 24, 35, 70, 74, 82, 117, 126). The hypothesis that host-generated molecules either derived from or functionally linked to phagocytosis instigate the death response also provides an explanation for the disparate fates of monocytes following the ingestion of *B. burgdorferi* or of *T. pallidum*. This dichotomy could reflect the divergent origins of the *B. burgdorferi* and *T. pallidum* phagosomes, most notably the latter's requirement for uptake by opsonic antibodies and Fc receptor signaling, which could modulate the signals by which the organelle informs the cytosol about the nature of its cargo (2, 27, 96).

Whether pathogen-induced apoptosis is harmful or beneficial to the host has been a considerable source of debate. Monocytes/macrophages are a major component of cellular infiltrates in *B. burgdorferi*-infected tissues (101) and are believed to be critical for clearance of spirochetes at the site of infection. Apoptosis of macrophages by ingested spirochetes could, therefore, be a parasitic suicide strategy for promoting survival and dissemination of bacteria that avoid uptake. On the other hand, apoptosis provides a means for the host to down-modulate the inflammatory response and limit the damage caused by proinflammatory bacterial constituents. *B. burgdorferi* is extremely susceptible to antibody-mediated killing (30). Here we showed that monocytes/macrophages have the

capacity to distinguish internalized viable bacteria from nonviable bacteria; induction of apoptosis would enable phagocytes to dispose of dead bacteria at the local site while minimizing collateral damage and further recruitment of inflammatory cells. Unlike monocytes, DCs, which also avidly ingest *B. burgdorferi* (88), were resistant to spirochete-induced apoptosis. DCs are highly enriched in erythema migrans infiltrates (101), where their resistance to apoptosis should help ensure their availability for generation of the adaptive response without which spirochetal clearance cannot occur (13, 103). Ingestion by DCs of antigen-containing blebs shed from apoptotic macrophages appears to be a primary mechanism for cross-presentation of antigens from bacteria that remain confined to the phagosome (5, 104, 125). This phenomenon could, therefore, explain the long-standing enigma of how LD spirochetes induce $CD8^+$ T cells (23, 38, 51, 64). The finding that *T. pallidum* did not induce a comparable cell death response is intriguing and points to the hitherto unappreciated divergence in the strategies by which *T. pallidum* and *B. burgdorferi* engage the immune systems of infected humans. This difference may be one more example of the syphilis spirochete's remarkable degree of adaptation to its obligate human host as a stealth pathogen (95).

ACKNOWLEDGMENTS

We thank Morgan La Vake and Cynthia Gonzalez for superb technical support, Carolina Bagnato for assistance with microscopy, and Christine Abreu for performing the qRT-PCR analyses.

This work was supported by Public Health Service grants AI-26756, AI-29735, and AI-38894 (to J.D.R.) and K23 AI-62439 (to J.C.S.) and by General Clinical Research Center grant M01RR06192 from the National Institutes of Health.

REFERENCES

1. **Adams, J. M.** 2003. Ways of dying: multiple pathways to apoptosis. Genes Dev. **17:**2481–2495.
2. **Aderem, A., and D. M. Underhill.** 1999. Mechanisms of phagocytosis in macrophages. Annu. Rev. Immunol. **17:**593–623.
3. **Akira, S., S. Uematsu, and O. Takeuchi.** 2006. Pathogen recognition and innate immunity. Cell **124:**783–801.
4. **Albee, L., B. Shi, and H. Perlman.** 2007. Aspartic protease and caspase 3/7 activation are central for macrophage apoptosis following infection with *Escherichia coli*. J. Leukoc. Biol. **81:**229–237.
5. **Albert, M. L., B. Sauter, and N. Bhardwaj.** 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature **392:**86–89.
6. **Albina, J. E., S. Cui, R. B. Mateo, and J. S. Reichner.** 1993. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. J. Immunol. **150:**5080–5085.
7. **Alexopoulou, L., V. Thomas, M. Schnare, Y. Lobet, J. Anguita, R. T. Schoen, R. Medzhitov, E. Fikrig, and R. A. Flavell.** 2002. Hyporesponsiveness to vaccination with *Borrelia burgdorferi* OspA in humans and in TLR1- and TLR2-deficient mice. Nat. Med. **8:**878–884.
8. **Aliprantis, A. O., D. S. Weiss, and A. Zychlinsky.** 2001. Toll-like receptor-2 transduces signals for NF-kappa B activation, apoptosis and reactive oxygen species production. J. Endotoxin Res. **7:**287–291.
9. **Aliprantis, A. O., R. B. Yang, M. R. Mark, S. Suggett, B. Devaux, J. D. Radolf, G. R. Klimpel, P. Godowski, and A. Zychlinsky.** 1999. Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. Science **285:**736–739.
10. **Aliprantis, A. O., R. B. Yang, D. S. Weiss, P. Godowski, and A. Zychlinsky.** 2000. The apoptotic signaling pathway activated by Toll-like receptor-2. EMBO J. **19:**3325–3336.
11. **Asch, E. S., D. I. Bujak, M. Weiss, M. G. Peterson, and A. Weinstein.** 1994. Lyme disease: an infectious and postinfectious syndrome. J. Rheumatol. **21:**454–461.
12. **Baran, J., K. Weglarczyk, M. Mysiak, K. Guzik, M. Ernst, H. D. Flad, and J. Pryjma.** 2001. Fas (CD95)-Fas ligand interactions are responsible for monocyte apoptosis occurring as a result of phagocytosis and killing of *Staphylococcus aureus*. Infect. Immun. **69:**1287–1297.
13. **Barthold, S. W., E. Hodzic, S. Tunev, and S. Feng.** 2006. Antibody-mediated disease remission in the mouse model of Lyme borreliosis. Infect. Immun. **74:**4817–4825.
14. **Behera, A. K., E. Hildebrand, S. Uematsu, S. Akira, J. Coburn, and L. T. Hu.** 2006. Identification of a TLR-independent pathway for *Borrelia burgdorferi*-induced expression of matrix metalloproteinases and inflammatory mediators through binding to integrin alpha 3 beta 1. J. Immunol. **177:**657–664.
15. **Bekeredjian-Ding, I., S. I. Roth, S. Gilles, T. Giese, A. Ablasser, V. Hornung, S. Endres, and G. Hartmann.** 2006. T cell-independent, TLR-induced IL-12p70 production in primary human monocytes. J. Immunol. **176:**7438–7446.
16. **Blaylock, M. G., B. H. Cuthbertson, H. F. Galley, N. R. Ferguson, and N. R. Webster.** 1998. The effect of nitric oxide and peroxynitrite on apoptosis in human polymorphonuclear leukocytes. Free Radic. Biol. Med. **25:**748–752.
17. **Bogdan, C.** 2001. Nitric oxide and the regulation of gene expression. Trends Cell Biol. **11:**66–75.
18. **Brightbill, H. D., D. H. Libraty, S. R. Krutzik, R. B. Yang, J. T. Belisle, J. R. Bleharski, M. Maitland, M. V. Norgard, S. E. Plevy, S. T. Smale, P. J. Brennan, B. R. Bloom, P. J. Godowski, and R. L. Modlin.** 1999. Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. Science **285:**732–736.
19. **Brown, J. P., J. F. Zachary, C. Teuscher, J. J. Weis, and R. M. Wooten.** 1999. Dual role of interleukin-10 in murine Lyme disease: regulation of arthritis severity and host defense. Infect. Immun. **67:**5142–5150.
20. **Brown, S. B., and J. Savill.** 1999. Phagocytosis triggers macrophage release of Fas ligand and induces apoptosis of bystander leukocytes. J. Immunol. **162:**480–485.
21. **Brumell, J. H., and S. Grinstein.** 2003. Role of lipid-mediated signal transduction in bacterial internalization. Cell. Microbiol. **5:**287–297.
22. **Brusca, J. S., A. W. McDowall, M. V. Norgard, and J. D. Radolf.** 1991. Localization of outer surface proteins A and B in both the outer membrane and intracellular compartments of *Borrelia burgdorferi*. J. Bacteriol. **173:**8004–8008.
23. **Busch, D. H., C. Jassoy, U. Brinckmann, H. Girschick, and H. I. Huppertz.** 1996. Detection of *Borrelia burgdorferi*-specific $CD8^+$ cytotoxic T cells in patients with Lyme arthritis. J. Immunol. **157:**3534–3541.
24. **Buttke, T. M., and P. A. Sandstrom.** 1994. Oxidative stress as a mediator of apoptosis. Immunol. Today **15:**7–10.
25. **Carpenter, S., and L. A. O'Neill.** 2007. How important are Toll-like receptors for antimicrobial responses? Cell. Microbiol. **9:**1891–1901.
26. **Casjens, S., N. Palmer, R. van Vugt, W. M. Huang, B. Stevenson, P. Rosa, R. Lathigra, G. Sutton, J. Peterson, R. J. Dodson, D. Haft, E. Hickey, M. Gwinn, O. White, and C. M. Fraser.** 2000. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. Mol. Microbiol. **35:**490–516.
27. **Chimini, G., and P. Chavrier.** 2000. Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. Nat. Cell Biol. **2:**E191–E196.
28. **Ciabattini, A., A. M. Cuppone, R. Pulimeno, F. Iannelli, G. Pozzi, and D. Medaglini.** 2006. Stimulation of human monocytes with the gram-positive vaccine vector *Streptococcus gordonii*. Clin. Vaccine Immunol. **13:**1037–1043.
29. **Coleman, J. L., and J. L. Benach.** 2003. The urokinase receptor can be induced by *Borrelia burgdorferi* through receptors of the innate immune system. Infect. Immun. **71:**5556–5564.
30. **Connolly, S. E., and J. L. Benach.** 2005. The versatile roles of antibodies in *Borrelia* infections. Nat. Rev. Microbiol. **3:**411–420.
31. **Cox, D. L., D. R. Akins, K. W. Bourell, P. Lahdenne, M. V. Norgard, and J. D. Radolf.** 1996. Limited surface exposure of *Borrelia burgdorferi* outer surface lipoproteins. Proc. Natl. Acad. Sci. USA **93:**7973–7978.
32. **Crandall, H., D. M. Dunn, Y. Ma, R. M. Wooten, J. F. Zachary, J. H. Weis, R. B. Weiss, and J. J. Weis.** 2006. Gene expression profiling reveals unique pathways associated with differential severity of Lyme arthritis. J. Immunol. **177:**7930–7942.
33. **Creagh, E. M., H. Conroy, and S. J. Martin.** 2003. Caspase-activation pathways in apoptosis and immunity. Immunol. Rev. **193:**10–21.
34. **Defosse, D. L., and R. C. Johnson.** 1992. In vitro and in vivo induction of tumor necrosis factor alpha by *Borrelia burgdorferi*. Infect. Immun. **60:**1109–1113.
35. **DeLeo, F. R.** 2004. Modulation of phagocyte apoptosis by bacterial pathogens. Apoptosis **9:**399–413.
36. **Dennis, V. A., A. Jefferson, S. R. Singh, F. Ganapamo, and M. T. Philipp.** 2006. Interleukin-10 anti-inflammatory response to *Borrelia burgdorferi*, the agent of Lyme disease: a possible role for suppressors of cytokine signaling 1 and 3. Infect. Immun. **74:**5780–5789.
37. **Desjardins, M., M. Houde, and E. Gagnon.** 2005. Phagocytosis: the convoluted way from nutrition to adaptive immunity. Immunol. Rev. **207:**158–165.
38. **Dong, Z., M. D. Edelstein, and L. J. Glickstein.** 1997. $CD8^+$ T cells are activated during the early Th1 and Th2 immune responses in a murine Lyme disease model. Infect. Immun. **65:**5334–5337.

39. **Eggers, C. H., M. J. Caimano, M. L. Clawson, W. G. Miller, D. S. Samuels, and J. D. Radolf.** 2002. Identification of loci critical for replication and compatibility of a *Borrelia burgdorferi* cp32 plasmid and use of a cp32-based shuttle vector for the expression of fluorescent reporters in the Lyme disease spirochaete. Mol. Microbiol. **43:**281–295.
40. **Eggers, C. H., M. J. Caimano, and J. D. Radolf.** 2006. Sigma factor selectivity in *Borrelia burgdorferi*: RpoS recognition of the ospE/ospF/elp promoters is dependent on the sequence of the −10 region. Mol. Microbiol. **59:**1859–1875.
41. **Ehrt, S., D. Schnappinger, S. Bekiranov, J. Drenkow, S. Shi, T. R. Gingeras, T. Gaasterland, G. Schoolnik, and C. Nathan.** 2001. Reprogramming of the macrophage transcriptome in response to interferon-γ and *Mycobacterium tuberculosis*: signaling roles of nitric oxide synthase-2 and phagocyte oxidase. J. Exp. Med. **194:**1123–1140.
42. **Embers, M. E., R. Ramamoorthy, and M. T. Philipp.** 2004. Survival strategies of *Borrelia burgdorferi*, the etiologic agent of Lyme disease. Microbes Infect. **6:**312–318.
43. **Feng, G., and N. Kaplowitz.** 2002. Mechanism of staurosporine-induced apoptosis in murine hepatocytes. Am. J. Physiol. Gastrointest. Liver Physiol. **282:**G825–G834.
44. **Fialkow, L., Y. Wang, and G. P. Downey.** 2007. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radic. Biol. Med. **42:**153–164.
45. **Fink, S. L., and B. T. Cookson.** 2005. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect. Immun. **73:**1907–1916.
46. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. Weidman, T. Utterback, L. Watthey, L. McDonald, P. Artiach, C. Bowman, S. Garland, C. Fujii, M. D. Cotton, K. Horst, K. Roberts, B. Hatch, H. O. Smith, and J. C. Venter.** 1997. Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi. Nature **390:**580–586.
47. **Gao, L., and K. Y. Abu.** 2000. Hijacking of apoptotic pathways by bacterial pathogens. Microbes Infect. **2:**1705–1719.
48. **Gavrilin, M. A., I. J. Bouakl, N. L. Knatz, M. D. Duncan, M. W. Hall, J. S. Gunn, and M. D. Wewers.** 2006. Internalization and phagosome escape required for *Francisella* to induce human monocyte IL-1B processing and release. Proc. Natl. Acad. Sci. USA **103:**141–146.
49. **Gebbia, J. A., J. L. Coleman, and J. L. Benach.** 2001. *Borrelia* spirochetes upregulate release and activation of matrix metalloproteinase gelatinase B (MMP-9) and collagenase 1 (MMP-1) in human cells. Infect. Immun. **69:**456–462.
50. **Giambartolomei, G. H., V. A. Dennis, B. L. Lasater, and M. T. Philipp.** 1999. Induction of pro- and anti-inflammatory cytokines by *Borrelia burgdorferi* lipoproteins in monocytes is mediated by CD14. Infect. Immun. **67:**140–147.
51. **Glickstein, L., M. Edelstein, and J. Z. Dong.** 2001. Gamma interferon is not required for arthritis resistance in the murine Lyme disease model. Infect. Immun. **69:**3737–3743.
52. **Glickstein, L. J., and J. L. Coburn.** 2006. Short report. Association of macrophage inflammatory response and cell death after *in vitro Borrelia burgdorferi* infection with arthritis resistance. Am. J. Trop. Med. Hyg. **75:**964–967.
53. **Godard, T., E. Deslandes, P. Lebailly, C. Vigreux, L. Poulain, F. Sichel, J. M. Poul, and P. Gauduchon.** 1999. Comet assay and DNA flow cytometry analysis of staurosporine-induced apoptosis. Cytometry **36:**117–122.
54. **Gorden, K. B., K. S. Gorski, S. J. Gibson, R. M. Kedl, W. C. Kieper, X. Qiu, M. A. Tomai, S. S. Alkan, and J. P. Vasilakos.** 2005. Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. J. Immunol. **174:**1259–1268.
55. **Haase, R., C. J. Kirschning, A. Sing, P. Schrottner, K. Fukase, S. Kusumoto, H. Wagner, J. Heesemann, and K. Ruckdeschel.** 2003. A dominant role of Toll-like receptor 4 in the signaling of apoptosis in bacteria-faced macrophages. J. Immunol. **171:**4294–4303.
56. **Hacker, G., S. Kirschnek, and S. F. Fischer.** 2006. Apoptosis in infectious disease: how bacteria interfere with the apoptotic apparatus. Med. Microbiol. Immunol. **195:**11–19.
57. **Hacker, H., C. Furmann, H. Wagner, and G. Hacker.** 2002. Caspase-9/-3 activation and apoptosis are induced in mouse macrophages upon ingestion and digestion of *Escherichia coli* bacteria. J. Immunol. **169:**3172–3179.
58. **Haile, W. B., J. L. Coleman, and J. L. Benach.** 2006. Reciprocal upregulation of urokinase plasminogen activator and its inhibitor, PAI-2, by *Borrelia burgdorferi* affects bacterial penetration and host-inflammatory response. Cell. Microbiol. **8:**1349–1360.
59. **Hazlett, K. R. O., T. J. Sellati, T. T. Nguyen, D. L. Cox, M. L. Clawson, M. J. Caimano, and J. D. Radolf.** 2001. The TprK protein of *Treponema pallidum* is periplasmic and is not a target of opsonic antibody or protective immunity. J. Exp. Med. **193:**1015–1026.
60. **Hirschfeld, M., C. J. Kirschning, R. Schwandner, H. Wesche, J. H. Weis, R. M. Wooten, and J. J. Weis.** 1999. Inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by Toll-like receptor 2. J. Immunol. **163:**2382–2386.
61. **Hueffer, K., and J. E. Galan.** 2004. *Salmonella*-induced macrophage death: multiple mechanisms, different outcomes. Cell. Microbiol. **6:**1019–1025.
62. **Into, T., K. Kiura, M. Yasuda, H. Kataoka, N. Inoue, A. Hasebe, K. Takeda, S. Akira, and K. Shibata.** 2004. Stimulation of human Toll-like receptor (TLR) 2 and TLR6 with membrane lipoproteins of *Mycoplasma fermentans* induces apoptotic cell death after NF-kappa B activation. Cell. Microbiol. **6:**187–199.
63. **Iwasaki, A., and R. Medzhitov.** 2004. Toll-like receptor control of the adaptive immune responses. Nat. Immunol. **5:**987–995.
64. **Jacobsen, M., D. Zhou, S. Cepok, S. Nessler, M. Happel, S. Stei, B. Wilske, N. Sommer, and B. Hemmer.** 2003. Clonal accumulation of activated $CD8^+$ T cells in the central nervous system during the early phase of neuroborreliosis. J. Infect. Dis. **187:**963–973.
65. **Jarvelainen, H. A., A. Galmiche, and A. Zychlinsky.** 2003. Caspase-1 activation by Salmonella. Trends Cell Biol. **13:**204–209.
66. **Kanneganti, T. D., N. Ozoren, M. Body-Malapel, A. Amer, J. H. Park, L. Franchi, J. Whitfield, W. Barchet, M. Colonna, P. Vandenabeele, J. Bertin, A. Coyle, E. P. Grant, S. Akira, and G. Nunez.** 2006. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. Nature **440:**233–236.
67. **Kanzler, H., F. J. Barrat, E. M. Hessel, and R. L. Coffman.** 2007. Therapeutic targeting of innate immunity with Toll-like receptor agonists and antagonists. Nat. Med. **13:**552–559.
68. **Kirschnek, S., S. Ying, S. F. Fischer, H. Hacker, A. Villunger, H. Hochrein, and G. Hacker.** 2005. Phagocytosis-induced apoptosis in macrophages is mediated by up-regulation and activation of the Bcl-2 homology domain 3-only protein Bim. J. Immunol. **174:**671–679.
69. **Klempner, M. S., L. T. Hu, J. Evans, C. H. Schmid, G. M. Johnson, R. P. Trevino, D. Norton, L. Levy, D. Wall, J. McCall, M. Kosinski, and A. Weinstein.** 2001. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N. Engl. J. Med. **345:**85–92.
70. **Kobayashi, S. D., K. R. Braughton, A. R. Whitney, J. M. Voyich, T. G. Schwan, J. M. Musser, and F. R. DeLeo.** 2003. Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. Proc. Natl. Acad. Sci. USA **100:**10948–10953.
71. **Koski, G. K., K. Kariko, S. Xu, D. Weissman, P. A. Cohen, and B. J. Czerniecki.** 2004. Cutting edge: innate immune system discriminates between RNA containing bacterial versus eukaryotic structural features that prime for high-level IL-12 secretion by dendritic cells. J. Immunol. **172:**3989–3993.
72. **LaFond, R. E., and S. A. Lukehart.** 2006. Biological basis for syphilis. Clin. Microbiol. Rev. **19:**29–49.
73. **Landmann, R., C. Ludwig, R. Obrist, and J. P. Obrecht.** 1991. Effect of cytokines and lipopolysaccharide on CD14 antigen expression in human monocytes and macrophages. J. Cell. Biochem. **47:**317–329.
74. **Laochumroonvorapong, P., S. Paul, K. B. Elkon, and G. Kaplan.** 1996. H_2O_2 induces monocyte apoptosis and reduces viability of *Mycobacterium avium-M. intracellulare* within cultured human monocytes. Infect. Immun. **64:**452–459.
75. **Lecoeur, H., E. Ledru, M. C. Prevost, and M. L. Gougeon.** 1997. Strategies for phenotyping apoptotic peripheral human lymphocytes comparing ISNT, annexin-V and 7-AAD cytofluorometric staining methods. J. Immunol. Methods **209:**111–123.
76. **Lee, M. S., and Y. J. Kim.** 2007. Signaling pathways downstream of pattern-recognition receptors and their cross talk. Annu. Rev. Biochem. **76:**447–480.
77. **Lin, M., and Y. Rikihisa.** 2004. *Ehrlichia chaffeensis* downregulates surface Toll-like receptors 2/4, CD14 and transcription factors PU.1 and inhibits lipopolysaccharide activation of NF-kappa B, ERK 1/2 and p38 MAPK in host monocytes. Cell. Microbiol. **6:**175–186.
78. **Lopez, M., L. M. Sly, Y. Luu, D. Young, H. Cooper, and N. E. Reiner.** 2003. The 19-kDa *Mycobacterium tuberculosis* protein induces macrophage apoptosis through Toll-like receptor-2. J. Immunol. **170:**2409–2416.
79. **Majno, G., and I. Joris.** 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. Am. J. Pathol. **146:**3–15.
80. **Mariathasan, S., and D. M. Monack.** 2007. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nat. Rev. Immunol. **7:**31–40.
81. **Mariathasan, S., D. S. Weiss, K. Newton, J. McBride, K. O'Rourke, M. Roose-Girma, W. P. Lee, Y. Weinrauch, D. M. Monack, and V. M. Dixit.** 2006. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature **440:**228–232.
82. **Marriott, H. M., F. Ali, R. C. Read, T. J. Mitchell, M. K. Whyte, and D. H. Dockrell.** 2004. Nitric oxide levels regulate macrophage commitment to apoptosis or necrosis during pneumococcal infection. FASEB J. **18:**1126–1128.
83. **Marriott, H. M., P. G. Hellewell, M. K. Whyte, and D. H. Dockrell.** 2007. Contrasting roles for reactive oxygen species and nitric oxide in the innate

response to pulmonary infection with *Streptococcus pneumoniae*. Vaccine **25:**2485–2490.
84. **Martinon, F., and J. Tschopp.** 2007. Inflammatory caspases and inflammasomes: master switches of inflammation. Cell Death Differ. **14:**10–22.
85. **McCaffrey, R. L., P. Fawcett, M. O'Riordan, K. D. Lee, E. A. Havell, P. O. Brown, and D. A. Portnoy.** 2004. A specific gene expression program triggered by Gram-positive bacteria in the cytosol. Proc. Natl. Acad. Sci. USA **101:**11386–11391.
86. **Monack, D. M., W. W. Navarre, and S. Falkow.** 2001. Salmonella-induced macrophage death: the role of caspase-1 in death and inflammation. Microbes Infect. **3:**1201–1212.
87. **Montgomery, R. R., and S. E. Malawista.** 1996. Entry of *Borrelia burgdorferi* into macrophages is end-on and leads to degradation in lysosomes. Infect. Immun. **64:**2867–2872.
88. **Moore, M. W., A. R. Cruz, C. J. LaVake, A. L. Marzo, C. H. Eggers, J. C. Salazar, and J. D. Radolf.** 2007. Phagocytosis of *Borrelia burgdorferi* and *Treponema pallidum* potentiates innate immune activation and induces gamma interferon production. Infect. Immun. **75:**2046–2062.
89. **Murray, R. Z., J. G. Kay, D. G. Sangermani, and J. L. Stow.** 2005. A role for the phagosome in cytokine secretion. Science **310:**1492–1495.
90. **Nadelman, R. B., J. Nowakowski, G. Forseter, N. S. Goldberg, S. Bittker, D. Cooper, M. Aguero-Rosenfeld, and G. P. Wormser.** 1996. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. Am. J. Med. **100:**502–508.
91. **Neumeister, B., M. Faigle, K. Lauber, H. Northoff, and S. Wesselborg.** 2002. *Legionella pneumophila* induces apoptosis via the mitochondrial death pathway. Microbiology **148:**3639–3650.
92. **Nilsen, N., U. Nonstad, N. Khan, C. F. Knetter, S. Akira, A. Sundan, T. Espevik, and E. Lien.** 2004. Lipopolysaccharide and double-stranded RNA up-regulate Toll-like receptor 2 independently of myeloid differentiation factor 88. J. Biol. Chem. **279:**39727–39735.
93. **Ozinsky, A., D. M. Underhill, J. D. Fontenot, A. M. Hajjar, K. D. Smith, C. B. Wilson, L. Schroeder, and A. Aderem.** 2000. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. Proc. Natl. Acad. Sci. USA **97:**13766–13771.
94. **Perskvist, N., M. Long, O. Stendahl, and L. Zheng.** 2002. *Mycobacterium tuberculosis* promotes apoptosis in human neutrophils by activating caspase-3 and altering expression of Bax/Bcl-xL via an oxygen-dependent pathway. J. Immunol. **168:**6358–6365.
95. **Radolf, J. D., and S. A. Lukehart.** 2006. Immunology of syphilis, p. 285–322. *In* J. D. Radolf and S. A. Lukehart (ed.), Pathogenic treponemes: cellular and molecular biology. Caister Academic Press, Norfolk, United Kingdom.
96. **Ravetch, J. V., and S. Bolland.** 2001. IgG Fc receptors. Annu. Rev. Immunol. **19:**275–290.
97. **Riedl, S. J., and Y. Shi.** 2004. Molecular mechanisms of caspase regulation during apoptosis. Nat. Rev. Mol. Cell Biol. **5:**897–907.
98. **Ruckdeschel, K., G. Pfaffinger, R. Haase, A. Sing, H. Weighardt, G. Hacker, B. Holzmann, and J. Heesemann.** 2004. Signaling of apoptosis through TLRs critically involves Toll/IL-1 receptor domain-containing adapter inducing IFN-beta, but not MyD88, in bacteria-infected murine macrophages. J. Immunol. **173:**3320–3328.
99. **Salazar, J. C., A. R. Cruz, C. D. Pope, L. Valderrama, R. Trujillo, N. G. Saravia, and J. D. Radolf.** 2007. *Treponema pallidum* elicits innate and adaptive cellular immune responses in skin and blood during secondary syphilis: a flow cytometric analysis. J. Infect. Dis. **195:**879–887.
100. **Salazar, J. C., C. D. Pope, M. W. Moore, J. Pope, T. G. Kiely, and J. D. Radolf.** 2005. Lipoprotein-dependent and -independent immune responses to spirochetal infection. Clin. Diagn. Lab. Immunol. **12:**949–958.
101. **Salazar, J. C., C. D. Pope, T. J. Sellati, H. M. Feder, Jr., T. G. Kiely, K. R. Dardick, R. L. Buckman, M. W. Moore, M. J. Caimano, J. G. Pope, P. J. Krause, and J. D. Radolf.** 2003. Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. J. Immunol. **171:**2660–2670.
102. **Samuels, D. S.** 1995. Electrotransformation of the spirochete *Borrelia burgdorferi*. Electrotransformation protocols for microorganisms. Methods Mol. Biol. **47:**253–259.
103. **Schaible, U. E., M. D. Kramer, C. Museteanu, G. Zimmer, H. Mossmann, and M. M. Simon.** 1989. The severe combined immunodeficiency (scid) mouse. A laboratory model for the analysis of Lyme arthritis and carditis. J. Exp. Med. **170:**1427–1432.
104. **Schaible, U. E., F. Winau, P. A. Sieling, K. Fischer, H. L. Collins, K. Hagens, R. L. Modlin, V. Brinkmann, and S. H. Kaufmann.** 2003. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. Nat. Med. **9:**1039–1046.
105. **Sellati, T. J., D. A. Bouis, R. L. Kitchens, R. P. Darveau, J. Pugin, R. J. Ulevitch, S. C. Gangloff, S. M. Goyert, M. V. Norgard, and J. D. Radolf.** 1998. *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins and synthetic lipopeptides activate monocytic cells via a CD14-dependent pathway distinct from that used by lipopolysaccharide. J. Immunol. **160:**5455–5464.
106. **Sellati, T. J., S. L. Waldrop, J. C. Salazar, P. R. Bergstresser, L. J. Picker, and J. D. Radolf.** 2001. The cutaneous response in humans to *Treponema pallidum* lipoprotein analogues involves cellular elements of both innate and adaptive immunity. J. Immunol. **166:**4131–4140.
107. **Smith, A. C., W. D. Heo, V. Braun, X. Jiang, C. Macrae, J. E. Casanova, M. A. Scidmore, S. Grinstein, T. Meyer, and J. H. Brumell.** 2007. A network of Rab GTPases controls phagosome maturation and is modulated by *Salmonella enterica* serovar *Typhimurium*. J. Cell Biol. **176:**263–268.
108. **Steere, A. C.** 2001. Lyme disease. N. Engl. J. Med. **345:**115–125.
109. **Steere, A. C., J. Coburn, and L. Glickstein.** 2004. The emergence of Lyme disease. J. Clin. Investig. **113:**1093–1101.
110. **Steere, A. C., and L. Glickstein.** 2004. Elucidation of Lyme arthritis. Nat. Rev. Immunol. **4:**143–152.
111. **Steere, A. C., W. Klitz, E. E. Drouin, B. A. Falk, W. W. Kwok, G. T. Nepom, and L. A. Baxter-Lowe.** 2006. Antibiotic-refractory Lyme arthritis is associated with HLA-DR molecules that bind a *Borrelia burgdorferi* peptide. J. Exp. Med. **203:**961–971.
112. **Steiner, T. S.** 2007. How flagellin and Toll-like receptor 5 contribute to enteric infection. Infect. Immun. **75:**545–552.
113. **Stow, J. L., A. P. Manderson, and R. Z. Murray.** 2006. SNAREing immunity: the role of SNAREs in the immune system. Nat. Rev. Immunol. **6:**919–929.
114. **Stuart, L. M., and R. A. Ezekowitz.** 2005. Phagocytosis: elegant complexity. Immunity **22:**539–550.
115. **Sutterwala, F. S., Y. Ogura, M. Szczepanik, M. Lara-Tejero, G. S. Lichtenberger, E. P. Grant, J. Bertin, A. J. Coyle, J. E. Galan, P. W. Askenase, and R. A. Flavell.** 2006. Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. Immunity **24:**317–327.
116. **Trinchieri, G., and A. Sher.** 2007. Cooperation of Toll-like receptor signals in innate immune defence. Nat. Rev. Immunol. **7:**179–190.
117. **Ulett, G. C., and E. E. Adderson.** 2005. Nitric oxide is a key determinant of group B streptococcus-induced murine macrophage apoptosis. J. Infect. Dis. **191:**1761–1770.
118. **Underhill, D. M., and A. Ozinsky.** 2002. Phagocytosis of microbes: complexity in action. Annu. Rev. Immunol. **20:**825–852.
119. **Underhill, D. M., A. Ozinsky, A. M. Hajjar, A. Stevens, C. B. Wilson, M. Bassetti, and A. Aderem.** 1999. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature **401:**811–815.
120. **Vermes, I., C. Haanen, and C. Reutelingsperger.** 2000. Flow cytometry of apoptotic cell death. J. Immunol. Methods **243:**167–190.
121. **Walz, J. M., H. Gerhardt, M. Faigle, H. Wolburg, and B. Neumeister.** 2000. *Legionella* species of different human prevalence induce different rates of apoptosis in human monocytic cells. APMIS **108:**398–408.
122. **Wooten, R. M., Y. Ma, R. A. Yoder, J. P. Brown, J. H. Weis, J. F. Zachary, C. J. Kirschning, and J. J. Weis.** 2002. Toll-like receptor 2 is required for innate, but not acquired, host defense to *Borrelia burgdorferi*. J. Immunol. **168:**348–355.
123. **Wooten, R. M., T. B. Morrison, J. H. Weis, S. D. Wright, R. Thieringer, and J. J. Weis.** 1998. The role of CD14 in signaling mediated by outer membrane lipoproteins of *Borrelia burgdorferi*. J. Immunol. **160:**5485–5492.
124. **Wooten, R. M., and J. J. Weis.** 2001. Host-pathogen interactions promoting inflammatory Lyme arthritis: use of mouse models for dissection of disease processes. Curr. Opin. Microbiol. **4:**274–279.
125. **Yrlid, U., and M. Jo Wick.** 2000. *Salmonella*-induced apoptosis of infected macrophages results in presentation of a bacteria-encoded antigen after uptake by bystander dendritic cells. J. Exp. Med. **191:**613–623.
126. **Zhang, B., J. Hirahashi, X. Cullere, and T. N. Mayadas.** 2003. Elucidation of molecular events leading to neutrophil apoptosis following phagocytosis: cross-talk between caspase 8, reactive oxygen species, and MAPK/ERK activation. J. Biol. Chem. **278:**28443–28454.
127. **Zychlinsky, A., M. C. Prevost, and P. J. Sansonetti.** 1992. *Shigella flexneri* induces apoptosis in infected macrophages. Nature **358:**167–169.

Editor: F. C. Fang