Screening for *Toxoplasma gondii*-Regulated Transcriptional Responses in Lipopolysaccharide-Activated Macrophages

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***Toxoplasma gondii*-infected macrophages are blocked in production of the proinflammatory cytokines interleukin-12 (IL-12) and tumor necrosis factor alpha (TNF- α) upon activation with lipopolysaccharide (LPS). Here, we used pathway-focused cDNA arrays to identify additional *T. gondii*-regulated transcriptional responses. Parasite infection decreased 57 (inclusive of IL-12 and TNF- α) and increased expression of 7 of 77 LPS-activated cytokine and cytokine-related genes. Interestingly, we found that the LPS-induced transcriptional response of the anti-inflammatory cytokine IL-10 was synergistically increased by *T. gondii*, results that we validated by conventional reverse transcription-PCR and enzyme-linked immunosorbent assay. Importantly, although the parasite exerted disparate effects in LPS-signaling leading to TNF- α versus IL-10 production, both responses required functional Toll-like receptor 4. We suggest that these effects represent parasite defense mechanisms to avoid or delay induction of antimicrobial activity and/or T-cell-mediated immunity during *Toxoplasma* infection.**

Toxoplasma gondii is an opportunistic intracellular parasite that asymptomatically infects 30 to 80% of the human population worldwide (14). In situations of immunodeficiency, such as occurs in AIDS patients, the parasite may emerge as a life-threatening pathogen (30, 31). *Toxoplasma* can also cause debilitating disease or death in congenitally infected infants (47). The course of infection in healthy individuals is characterized by an acute phase associated with dissemination of rapidly dividing tachyzoites capable of invading virtually all nucleated cells. This is followed by a long-term chronic phase, which correlates with the rise in host adaptive immunity. Infection at this stage is associated with parasite differentiation into bradyzoites that form quiescent cysts deep within tissues of the central nervous system and skeletal muscle (14).

Macrophages (M ϕ) are key components of innate immunity. These cells are proficient generalists that display both microbicidal and immunoregulatory properties (42). M ϕ neutralize pathogens through phagocytosis and production of nitric oxide and reactive oxygen intermediates (1, 33). More recently, gamma interferon (IFN- γ)-regulated p47 GTPases have emerged as potent effectors of microbial killing in cells such as M ϕ (9, 54). Although incapable of migrating from infected tissues to lymph nodes, M ϕ possess the ability to function as competent antigen presenting cells through expression of costimulatory molecules and major histocompatibility complex (MHC) class II proteins. In addition, M ϕ produce high amounts of proinflammatory cytokines including TNF- α , IL-6 and IL-12, as well as anti-inflammatory cytokines such as IL-10.

We, and others, found that *T. gondii* actively interferes with M ϕ function during intracellular infection (13, 50). The parasite is a potent suppressor of lipopolysaccharide (LPS) initiated signaling that leads to interleukin-12 (IL-12) and tumor

necrosis factor alpha (TNF- α) production (8, 10). IFN- γ -stimulated MHC class II upregulation and nitric oxide production are also suppressed in infected M ϕ (26–28). Simultaneously, *Toxoplasma gondii* disables nuclear factor (NF)- κ B, signal transducer and activator of transcription 1 (STAT1), and mitogen-activated protein kinase (MAPK) intracellular signaling pathways (10, 23, 29, 51). Recently, we found that parasite-induced activation of STAT3 plays an important role in suppression of lipopolysaccharide (LPS)-induced TNF- α and IL-12 production (11). *Toxoplasma* suppression of macrophage function may be a means to avoid host anti-microbial effector function, or it may be a strategy to prevent hyper-inflammatory responses that can lead to host (and therefore parasite) death.

To date, our studies have focused on LPS-triggered Toll-like receptor 4 (TLR4) signaling leading to IL-12 and TNF- α production. Here, we asked whether the parasite mediates global shut-down in TLR4-initiated signaling, or whether some LPS-inducible genes escape suppression by *T. gondii*. We show that the parasite blocks most LPS-inducible cytokine and cytokine-related genes. Nevertheless, a small number of genes, most prominently IL-10, escape suppression by the parasite.

MATERIALS AND METHODS

Mice. Female C57BL/6, C3H/HeJ, and C3H/HeN (C3H/HeOuJ) mice between 6 and 8 weeks old were purchased from Jackson Laboratory (Bar Harbor, ME). The animals were housed under specific-pathogen-free conditions at the Cornell University of Veterinary Medicine Animal Facility, which is accredited by the Association for Assessment and Accreditation of Laboratory Care.

Parasites. Tachyzoites of the virulent *Toxoplasma gondii* RH strain and the transgenic RH expressing yellow fluorescent protein (YFP-RH; kindly provided by D. Roos, University of Pennsylvania) were maintained by biweekly passage on human foreskin fibroblast monolayers in fibroblast medium consisting of Dulbecco's modified Eagle's medium (DMEM; Mediatech Inc., Herndon, VA), 1% heat-inactivated bovine growth serum (HyClone, Logan, UT), penicillin (100 U/ml; Invitrogen Life Technologies; Grand Island, NY), and streptomycin (0.1 mg/ml; Invitrogen).

Macrophage preparation. Bone marrow-derived macrophages (BMDM ϕ) were prepared with flushed femur and tibia bone marrow cells cultured in M ϕ medium consisting of DMEM (Mediatech), 10% heat-inactivated bovine growth serum (HyClone), 0.1 mM nonessential amino acids (HyClone), 1 mM sodium pyru-

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TABLE 1. Primers used for RT-PCR

Transcript	Primer ^a	Reference
CD83	5'-CAGGATGCCCCAAGGAAGCTAC-3' 5'-GGCAITTCAGGCACACTGATCTGA-3'	56
GAPDH	5'-ACCACAGTCCATGCCATCAC-3' 5'-TCCACCACCTGTTGCTGTA-3'	25
IL-1 β	5'-GAGCCCATCCTCTGTGACTCAT-3' 5'-GCTGATGTACCACTTGGGGAAC-3'	UniSTS 143542
IL-6	5'-CGTGGAAATGAGAAAAGATTGTC-3' 5'-TGCTTAGGCATAACGCACTAGGT-3'	20
IL-10	5'-ATGCCTGGCTCAGCACTGCTAT-3' 5'-GCTCTTATTTTCACAGGGGAGAA-3'	44
IL-12p40	5'-CGTGCTCATGGCTGGTGCAAAG-3' 5'-GAACACATGCCCACTTGCTG-3'	4
TNF- α	5'-CAGCCTCTTCTCATTCTGCTTG-3' 5'-CTGGAAGACTCCTCCAGGTATAT-3'	20

^a Primer sequences used for RT-PCR, with the sense primer followed by the antisense primer.

vate, penicillin (100 U/ml), streptomycin (0.1 mg/ml), and 20% L929 cell supernatant (as a source of M ϕ colony-stimulating factor; cells from the American Type Culture Collection, Manassas, VA). Products were purchased from Invitrogen unless stated otherwise. Cells were fed once with fresh M ϕ medium on the third day. On day 5, nonadherent cells were discarded, adherent cells were washed in calcium- and magnesium-free phosphate-buffered saline (Mediatech), and replated in D10 medium consisting of DMEM (Mediatech), 10% heat-inactivated bovine growth serum (HyClone), 0.1 mM nonessential amino acids (HyClone), 1 mM sodium pyruvate, 1 mM HEPES, penicillin (100 U/ml), and streptomycin (0.1 mg/ml).

Experimental setup. BMM ϕ were plated at 2.5×10^6 cells per well in six-well tissue culture plates (Falcon, Franklin Lakes, NJ). Each independent experiment consisted of a BMM ϕ medium control, cells infected with RH tachyzoites (6:1 ratio of parasites to cells), cells stimulated with 100 ng/ml of ultrapure LPS from *Salmonella minnesota* (List Biological Laboratories, Campbell, CA), and cells infected with tachyzoites (6:1 ratio of parasites to cells) and subsequently subjected to LPS stimulation. In some experiments, cells were stimulated with Pam3CSK4 (InvivoGen, San Diego, CA). Plates containing cells and parasites were briefly centrifuged to synchronize tachyzoite and M ϕ contact. At the termination of the experiment, culture supernatants and cells were collected for cytokine enzyme-linked immunosorbent assay (ELISA) and total RNA isolation, respectively.

Gene array analysis. Total cellular RNA was isolated using RNeasy mini kits (QIAGEN, Valencia, CA). The quality of the RNA was assessed both by $A_{260}:A_{280}$ ratio and visualization on an agarose gel. The commercial pathway-focused cDNA arrays (MM-604, mouse Dendritic and antigen presenting cell gene array, and MM-105, mouse inflammatory cytokines and receptors gene array) were purchased from SuperArray Bioscience Corp. (Frederick, MD), and analyses were performed using a chemiluminescence-based detection system according to the manufacturer's recommendations. Images were captured on X-ray film (Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom). Image data sets were scanned and analyzed using ScanAlyze, Microsoft Excel, and GEArray Analyzer software. Background adjustment was performed by subtracting the lowest measured value on the membrane from the values of all genes.

The data were subsequently normalized against the positive control housekeeping gene glyceraldehyde-3-phosphate dehydrogenase to obtain the processed data sets. Each array was performed at least twice to ensure reproducibility of the results. Average fold changes were calculated as the normalized ratio of average experimental processed data sets divided by the average medium control processed data sets. Thresholds were set to select for genes up-regulated by LPS treatment alone by twofold or more. Fold RH-induced repression was then calculated using the average ratio of LPS fold difference divided by RH plus LPS fold difference.

RT-PCR. RNA was isolated following the RNeasy mini handbook (QIAGEN). cDNA was prepared and reverse transcription (RT)-PCR was performed as described (4), using the following amplification protocol: Denaturation at 95°C for 3 min; 30 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min; final extension at 72°C for 7 min. The primer sequences used are shown in Table 1.

Cytokine ELISA. IL-12p40 was measured by capture ELISA as described elsewhere (4). IL-6, IL-10, and TNF- α were measured using a commercial kit in accordance with the manufacturer's recommendations (BD Biosciences, San Diego, CA).

Flow cytometry. To analyze BMM ϕ tumor necrosis factor receptor 2 (TNFR2) (Tnfrsf1b) and CD83 surface expression, YFP-RH tachyzoites were employed for infections using a 1.5:1 ratio of parasites to cells, and M ϕ were collected for flow cytometric analysis 18 and 24 h, respectively, after LPS triggering. The cells were preincubated in flow cytometry buffer (10% normal mouse serum, 1% bovine serum albumin, 0.1% NaN₃) for 15 min at 4°C, then stained with optimal concentrations of allophycocyanin-conjugated anti-F4/80 in combination with phycoerythrin-conjugated antisera specific for TNFR2 (BD Biosciences) and CD83 (eBioscience, San Diego, CA). Data were acquired on a FACSCalibur flow cytometer (10,000 events per sample), and analyzed with CellQuest software (BD Immunocytometry Systems, San Jose, CA).

RESULTS

Pathway-specific gene array reveals *Toxoplasma*-mediated down-regulation of most, but not all LPS-induced cytokine-related genes in BMM ϕ . Previous studies showed that *Toxoplasma* infection blocks LPS-induced TNF- α and IL12p40 secretion when supernatants were collected 6 h poststimulation (10). Here we employed pathway-specific arrays to determine the extent to which other LPS-induced genes were affected by parasite infection, employing the experimental set-up shown in Fig. 1. A pathway-focused array allows for rapid determination of mRNA expression levels of specific genes of interest. We used two different pathway-specific arrays, screening a total of 218 cytokine-related genes for *Toxoplasma*-regulated transcriptional responses in LPS-activated BMM ϕ . While these arrays are extremely useful in rapidly determining patterns of gene induction, they cannot be assumed to directly indicate actual fold changes.

Stimulation with LPS reproducibly induced a twofold or more induction of 77 genes relative to expression in medium. Preinfection with *Toxoplasma* resulted in parasite-induced repression of 57 genes relative to expression in LPS. These genes encode cytokines, chemokines, surface receptors, and proteins involved in antigen uptake, antigen presentation and signal transduction (Table 2). Among the genes whose LPS-induced induction was down regulated by parasite infection were

FIG. 1. Schematic representation of experimental setup. Adherent BMM ϕ were subjected to four treatments. Med, cells were cultured in medium for a total of 8 h. RH, cells were infected with RH strain tachyzoites (6:1 ratio of parasites to cells, resulting in >90% infection) and then cultured for an additional 6 h. LPS, cells were stimulated with LPS (100 ng/ml) for a total of 4 h. RH+LPS, cells were infected with RH (6:1 ratio of parasites to cells) and then 2 h later subjected to 4 h LPS (100 ng/ml) stimulation. At the termination of the experiment, culture supernatants and cells were collected for cytokine ELISA and gene array/RT-PCR analyses, respectively.

TABLE 2. LPS-induced genes down-regulated by *T. gondii*

Functional gene grouping	Gene	GenBank no.	Expt repeats	Avg difference ^a (fold)			RH-induced repression ^e (fold)
				LPS ^b	RH ^c	RH+LPS ^d	
Cytokines, cytokine receptors, & related genes	<i>Ifna1</i>	NM_010502	2	5.3	2.1	2.8	1.9
	<i>Ifnb</i>	NM_010510	2	2.6	1.6	1.3	2.0
	<i>Ifngr</i>	NM_010511	2	2.2	1.3	1.2	1.8
	<i>Il2</i>	NM_008366	4	2.1	1.1	1.0	2.1
	<i>Il6</i>	NM_031168	4	4.9	1.6	4.0	1.2
	<i>Il12b</i>	NM_008352	4	3.6	1.0	1.7	2.1
	<i>Il15</i>	NM_008357	4	3.2	0.8	1.6	2.0
	<i>Il15ra</i>	NM_008358	2	6.2	1.8	2.5	2.5
	<i>Tgfb2</i>	NM_009367	2	2.6	2.9	2.1	1.2
	<i>Tnf</i>	NM_013693	4	6.4	1.6	2.0	3.2
	<i>Tnfrsf1a</i>	NM_011609	2	2.2	1.3	1.2	1.8
	<i>Tnfrsf1b</i>	NM_011610	2	4.5	2.3	2.9	1.6
	<i>Tnfrsf5</i>	NM_011611	2	6.6	1.5	2.7	2.4
	<i>Tnfrsf6</i>	NM_007987	2	5.0	1.9	2.0	2.5
	<i>Tnfsf4</i>	NM_009452	2	2.9	2.4	0.9	3.2
	<i>Tnfsf11</i>	NM_011613	2	2.6	2.2	1.3	2.0
	<i>Tnfsf14</i>	NM_019418	2	2.7	2.0	0.9	3.0
Chemokines & receptors	<i>Ccl2</i>	NM_011333	2	26.0	19.0	20.0	1.3
	<i>Ccl3</i>	NM_011337	4	2.6	1.0	1.7	1.5
	<i>Ccl5</i>	NM_013653	4	6.1	1.3	2.5	2.4
	<i>Ccl7</i>	NM_013654	2	4.0	3.0	2.6	1.5
	<i>Ccl8</i>	NM_021443	2	3.5	2.7	2.5	1.4
	<i>Ccl12</i>	NM_011331	2	3.0	1.8	1.7	1.8
	<i>Ccl22</i>	NM_009137	4	2.8	1.5	2.4	1.2
	<i>Cx3cl1</i>	NM_009142	4	2.3	1.6	1.7	1.4
	<i>Cxcl2</i>	NM_009140	2	3.6	1.7	2.1	1.7
	<i>Cxcl10</i>	NM_021274	2	14.9	2.2	4.7	3.2
	<i>Cxcl11</i>	NM_019494	2	2.4	1.5	1.3	1.8
	<i>Cxcl13</i>	NM_018866	2	2.6	1.1	1.0	2.6
	<i>Cxcl16</i>	NM_023158	2	4.0	1.8	1.8	2.2
	<i>Xcl1</i>	NM_008510	2	2.6	1.3	1.2	2.2
Antigen presentation	<i>H2-DMA</i>	NM_010386	2	2.6	1.9	1.5	1.7
	<i>H2-DMb2</i>	NM_010388	2	2.5	1.2	0.7	3.6
Cell adhesion molecules	<i>Cd44</i>	XM_130536	2	2.4	0.9	2.0	1.2
	<i>Icam1</i>	NM_010493	2	4.7	1.8	2.1	2.2
	<i>Itgam</i>	NM_008401	2	2.4	2.0	1.3	1.8
	<i>Itgb2</i>	NM_008404	2	4.3	6.1	2.5	1.7
	<i>Vcl</i>	NM_009502	2	2.7	1.4	1.1	2.5
Transcription factors	<i>Nfkb2</i>	NM_019408	2	2.7	1.5	1.2	2.3
	<i>Rela</i>	NM_009045	2	2.3	1.3	1.8	1.3
	<i>Relb</i>	NM_009046	2	5.3	3.0	4.6	1.2
Other	<i>Cflar</i>	NM_009805	2	6.7	1.5	2.7	2.5
	<i>Dcm2-Rik</i>	NM_027151	2	2.2	1.5	1.3	1.7
	<i>Gbp2</i>	NM_010260	2	11.9	3.6	5.7	2.1
	<i>Gbp3</i>	NM_018734	2	6.4	1.2	3.5	1.8
	<i>Gpx4</i>	NM_008162	2	3.8	1.6	1.7	2.2
	<i>Igsf6</i>	NM_030691	2	3.5	1.5	1.7	2.1
	<i>Ifi204</i>	NM_008329	2	3.7	0.8	2.2	1.7
	<i>Ifi2</i>	NM_008332	2	6.5	1.5	1.9	3.4
	<i>Ifi3</i>	NM_010501	2	3.9	2.6	1.9	2.1
	<i>Isg12</i>	NM_015783	2	3.3	2.1	2.4	1.4
	<i>Mlp</i>	NM_010807	2	3.2	1.4	2.1	1.5
	<i>Mx1</i>	NM_010846	2	9.6	1.8	3.7	2.6
	<i>Mx2</i>	NM_013606	2	5.8	2.6	3.7	1.6
	<i>S100a4</i>	NM_011311	2	2.5	1.7	1.0	2.5
	<i>Tap1</i>	NM_013683	2	3.8	1.8	2.8	1.4
	<i>Tlr9</i>	NM_031178	2	2.5	1.4	1.5	1.7

^a Relative to expression in medium.^b 100 ng/ml.^c 6:1 ratio tachyzoites to cells.^d Preinfection with tachyzoites (6:1 ratio parasites to cells) followed by LPS (100 ng/ml).^e Relative to expression of gene in LPS, i.e., LPS/(RH+LPS).

TABLE 3. LPS-induced genes not affected by *T. gondii*

Functional gene grouping	Gene	GenBank no.	Expt repeats	Avg difference (fold) ^a			RH-induced effect (fold) ^e
				LPS ^b	RH ^c	RH+LPS ^d	
Cytokines, cytokine receptors, & related genes	<i>Il1b</i>	NM_008361	4	5.1	3.5	5.7	0.9
	<i>Il8rb</i>	NM_009909	2	3.0	2.5	3.1	1.0
	<i>Il11ra1</i>	NM_010549	2	2.0	2.2	2.2	0.9
	<i>Tgfa</i>	NM_031199	4	2.1	2.3	1.9	1.1
Chemokines & receptors	<i>Ccl4</i>	NM_013652	2	2.6	2.3	2.5	1.0
	<i>Ccl17</i>	NM_011332	4	2.1	2.0	2.4	0.9
Other	<i>Cd47</i>	NM_010581	2	3.3	1.9	3.7	0.9
	<i>Cd86</i>	NM_019388	2	2.4	1.8	2.9	0.8
	<i>Cst7</i>	NM_009977	2	2.8	1.5	2.6	1.1
	<i>Fgls</i>	NM_025378	2	2.4	1.8	2.2	1.1
	<i>Fscn3</i>	NM_019569	2	2.1	1.7	1.9	1.1
	<i>Sod2</i>	NM_013671	2	3.0	1.9	3.0	1.0
	<i>Thr2</i>	NM_011905	2	2.3	1.0	2.2	1.0

^a See Table 2, footnotes a to e.

TNF- α and IL-12p40, confirming previous results that assessed these cytokines by ELISA and RNase protection assay (10).

Another group of LPS-induced genes displayed little or no dependence upon RH infection (Table 3). There were 13 members of this group, including the genes for CD86, Toll-like receptor 2, and NF- κ B family member RelB. Interestingly, we identified a small group of seven LPS-induced genes whose expression was increased by *Toxoplasma* infection (Table 4). It is notable that the anti-inflammatory cytokine IL-10 is a member of this group.

In sum, the majority (74%) of LPS-induced genes were down regulated by *T. gondii* preinfection. Nevertheless, 17% of LPS-induced genes were unaffected and 9% of genes analyzed were up-regulated by the parasite. These data indicate that while the suppressive effects of *Toxoplasma* on LPS-induced responses are profound, they are not global.

Validation of pathway-specific gene arrays. Since *Toxoplasma* infection blocks LPS-stimulated BMM ϕ IL-12p40 and TNF- α production (10), we used these genes as references for our validation. As shown by RT-PCR analysis in Fig. 2A, mRNA levels for IL-6 were decreased in *T. gondii*-preinfected LPS-triggered culture although not to the extent observed for IL-12p40 and TNF- α . In contrast, IL-10 and CD83 transcripts

were increased by *Toxoplasma* infection. Gene transcripts typified by IL-1 β were unaffected by RH preinfection (Fig. 2A).

Next, we further validated IL-6 and IL-10 results using cytokine ELISA. As shown in Fig. 2B, LPS-induced IL-6 secretion was inhibited by *Toxoplasma* infection. This pattern parallels closely the effects of RH infection on IL-12p40 and TNF- α . In striking contrast, RH preinfection followed by LPS-triggering induced higher levels of IL-10 relative to stimulation by LPS alone (Fig. 2B).

We next performed flow cytometric analysis to validate the influence of *T. gondii* on LPS-induced expression of CD83 and TNFR2. In these experiments we employed transgenic RH strain parasites expressing YFP. As shown in Fig. 3A, LPS (red line) induced up-regulation of surface TNFR2 relative to medium alone (black line). While YFP-RH infection (green line) induced up-regulation of this receptor, expression levels were lower than that resulting from LPS stimulation. In addition, parasite infection followed by LPS stimulation (blue line) decreased TNFR2 expression levels relative to LPS alone. These data closely reflect the results obtained in the pathway-specific gene array (*Tnfrsf1b* in Table 2).

The CD83 gene emerged as one of the few whose expression levels were increased by *Toxoplasma* infection (Table 4). In

TABLE 4. LPS-induced genes up-regulated by *T. gondii*

Functional gene repeats	Gene	GenBank no.	Expt repeats	Avg difference (fold) ^a			RH-induced up-regulation (fold) ^e
				LPS ^b	RH ^c	RH+LPS ^d	
Interleukins & receptors	<i>Il1r1</i>	NM_008362	2	2.6	3.5	4.1	1.6
	<i>Il5</i>	NM_010558	2	3.7	5.8	5.3	1.4
	<i>Il10</i>	NM_010548	4	2.8	2.0	4.2	1.5
Chemokines—receptors	<i>Ccr7</i>	NM_007719	4	3.1	2.9	3.8	1.2
Chemokines—subfamily A (Cys-Cys)	<i>Ccl24</i>	NM_019577	2	2.4	8.8	12.3	5.1
Antigen uptake	<i>Prg</i>	NM_011157	2	2.3	1.7	2.8	1.2
Antigen presentation	<i>Cd83</i>	NM_009856	2	3.6	2.5	4.7	1.3

^a See Table 2, footnotes a to d.^e Relative to expression of gene in LPS, i.e., (RH + LPS)/LPS.

FIG. 2. Validation of pathway-specific gene array results. A, IL-12p40, TNF- α and IL-6 gene transcripts induced by LPS were decreased by preinfection with *Toxoplasma* as assessed by RT-PCR analysis. In contrast, levels of LPS-induced IL-10 and CD83 transcripts were increased further by preinfection. Gene transcripts typified by IL-1 β were unaffected by RH preinfection. B, Cytokine ELISA confirmed the down-regulatory effects of *Toxoplasma* on LPS-induced release of IL-12p40, TNF- α , and IL-6. In contrast, LPS-induced IL-10 release was increased by preinfection with *T. gondii*. L, stimulation with LPS; R, infection with RH strain tachyzoites; RL, RH preinfection followed by LPS stimulation; M, cells cultured in medium alone. Each experiment was repeated three times with similar results.

Fig. 3B, we assessed expression levels by flow cytometry. In contrast to TNFR2 expression, *Toxoplasma* infection did not suppress LPS-induced CD83 expression (green versus blue lines). Indeed, *T. gondii* alone induced significantly higher levels of CD83 relative to LPS (green versus red lines). Infection plus LPS induced a CD83 intermediate population and parasite alone stimulated higher CD83 expression compared to LPS. Nevertheless, the basic pattern (lack of parasite-induced CD83 suppression) also applied to the flow cytometry-based analysis.

***T. gondii* infection up-regulates LPS-induced IL-10 production.** The data in Table 4 and Fig. 2 suggested that *Toxoplasma* increased LPS-induced IL-10 without itself inducing this cytokine. Figure 4A shows the ability of the parasite to increase LPS-induced IL-10 over a range of multiplicities of infection. Inasmuch as LPS-induced TNF- α production is simultaneously suppressed in a parasite dose-dependent manner (Fig. 4B), it seems likely that inhibition of proinflammatory mediators by *Toxoplasma* is responsible for increased levels of IL-10.

LPS-induced TNF- α and IL-10 cytokine production depends upon functional Toll-like receptor 4. The major receptor for LPS on the cell surface is Toll-like receptor 4. Nevertheless, other cell surface molecules, such as scavenger receptors, also bind LPS (55). The finding that *T. gondii* suppresses LPS-induced signaling leading to cytokines such as TNF- α , yet does not block endotoxin-triggered signaling resulting in IL-10 release, raised the possibility that distinct LPS receptors were involved in each response. To address this question, BMM ϕ from C3H/HeN (functional TLR4) and C3H/HeJ (nonfunc-

FIG. 3. Divergent effects of *T. gondii* on LPS-induced expression of TNFR2 and CD83 in infected M ϕ . Cells were preinfected with YFP-expressing RH tachyzoites. Two hours later, cells were subjected to LPS triggering and then collected for assessment of TNFR2 (A) and CD83 (B) expression 18 and 24 h later, respectively. F4/80-positive infected cells were gated for analysis based upon YFP expression. Black line, cells in medium alone; red line, cells stimulated with LPS; blue line, cells infected with RH-YFP; green line, infected cells subjected to LPS triggering. This experiment was repeated twice with the same result.

FIG. 4. *Toxoplasma* infection exerts disparate effects on LPS-induced IL-10 and TNF- α production. M ϕ were preinfected and subjected to LPS stimulation and cytokine measurement as described for Fig. 1. R, RH alone; L, LPS alone; RL, preinfection followed by LPS triggering. This experiment was repeated three times with similar results.

tional TLR4) were subjected to LPS stimulation with and without RH preinfection. Although the parasite continued to exert disparate effects on LPS signaling leading to TNF- α and IL-10 production on a C3H/HeN genetic background, both LPS-induced responses required functional TLR4 as shown by the complete lack of response in C3H/HeJ M ϕ (Fig. 5). In contrast, TLR1/2 agonist Pam3CSK4 elicited TNF- α and IL-10 from both C3H/HeN and C3H/HeJ BMM ϕ .

DISCUSSION

Macrophages infected with *T. gondii* are unable to produce IL-12 or TNF- α when subjected to triggering through TLR4 (10). Infected M ϕ also display defects in NF- κ B nuclear translocation and MAPK activation upon LPS stimulation (10, 23, 51). Recently, we found that parasite-directed STAT3 activation in host cells plays a major role in the ability of *Toxoplasma* to suppress IL-12 and TNF- α (11). The down-modulatory effects of *T. gondii* appear to extend beyond M ϕ , insofar as infection of mouse bone marrow-derived dendritic cells is associated with inhibition of maturation and suppression of LPS-induced TNF- α and IL-12 (39).

Here, we show that among a panel of cytokine and cytokine-related genes, *Toxoplasma* inhibits most, but not all, responses induced by LPS. In particular, IL-10 emerged as a cytokine whose LPS-induced expression was not prevented by parasite infection. Interestingly, *Toxoplasma* not only failed to block LPS-induced IL-10, but actually increased levels of this cytokine while itself failing to induce IL-10. Because the parasite clearly downregulates a wide spectrum of proinflammatory mediators, we think the most likely explanation for this pattern of IL-10 expression is parasite-induced suppression of an IL-10 down-regulatory mediator whose identity is presently unknown. Alternatively, it is possible that *Toxoplasma* directly influences LPS-triggered IL-10.

The issue of whether *Toxoplasma* modulates the pattern of cytokine production induced by other TLRs in a pattern similar to LPS/TLR4, or other stimuli such as CD40L, is currently under investigation. Also currently under investigation is whether *Toxoplasma* strain virulence impacts the pattern of LPS-induced genes.

FIG. 5. LPS-triggered signaling leading to TNF- α and IL-10 cytokine production proceeds through TLR4. BMM ϕ from C3H/HeN (functional TLR4) and C3H/HeJ (nonfunctional TLR4) mice were subjected to LPS stimulation with and without RH strain tachyzoite preinfection as described for Fig. 1. R, RH alone; L, LPS alone; RL, preinfection followed by LPS triggering; M, cells cultured in medium alone; Pa, Pam3CSK4. This experiment was repeated three times with the same result.

Although TLR4 serves as the major receptor that mediates the biological effects of LPS, other surface molecules can bind to this bacterial molecule. For example, β -integrins, P- and L-selectin, and a class A scavenger receptor have each been reported to bind LPS (18, 34, 35, 40). Therefore, it was possible that the subsets of LPS-inducible responses not suppressed by *Toxoplasma* were mediated by TLR4-independent signaling. This was not the case for IL-10, because LPS failed to induce this cytokine in the absence of functional TLR4.

Signaling pathways mediated by TLR are currently the subject of intense investigation (15, 53). For TLR4, this involves both MyD88-dependent and MyD88-independent pathways. Surprisingly, recent data indicate that LPS induction of M ϕ gene expression predominantly uses MyD88-independent signaling (3). Nevertheless, the MyD88-dependent transduction pathway is critical for induction of proinflammatory cytokines such as TNF- α and IL-12, as well as IL-10 (3). Therefore, the inability of *T. gondii* to down-regulate LPS-induced IL-10, while simultaneously repressing other cytokines and chemokines, cannot be explained by a termination of MyD88-dependent TLR signaling that leaves MyD88-independent responses intact.

LPS-inducible control of promoters driving expression of proinflammatory genes has been found to be dependent upon a similar set of transcription factors, including Rel family members AP-1 and C/EBP (2, 6, 17, 45, 48). Much less is known about LPS induction of anti-inflammatory cytokines. For IL-10, while MyD88-dependent signaling is required (3), transcriptional control appears to be fundamentally different from induction of proinflammatory genes such as IL-12 and TNF- α .

Protein tyrosine kinases and protein kinase C, as well as elevated cyclic AMP are reported to be required in LPS-induced IL-10 induction in mouse cell lines (21, 32, 41). In addition, IL-1 receptor-associated kinase (IRAK)1-mediated STAT3 activation has been implicated in LPS-driven IL-10 gene induction (19). Transcription factor Sp1 is another molecule implicated in LPS-induced IL-10, and in the human monocyte line THP-1 activation of this transcription factor depends upon p38 MAPK (7, 16). Interestingly, and in striking contrast to LPS-induced TNF- α and IL-12, overexpression studies employing I κ B α and chemical inhibition of Rel proteins suggests that NF- κ B signaling is not required for IL-10 induction (5, 43).

Despite the fact that *Toxoplasma* blocks IL-12 production triggered through TLR4, the RH parasite strain used here eventually induces the cytokine in BMM ϕ , though at lower levels and with delayed kinetics (24). The nature of the parasite-induced IL-12 induction is not yet clear. The absence of a requirement for c-Rel that is implicated in LPS-induced IL-12 production (36, 48), as well as the lack of involvement of other NF κ B family members (37), suggests an atypical activation pathway may be involved. This concept is further reinforced by the finding that, while p38 MAPK activation is required for parasite-induced IL-12, activation of this MAPK is dependent upon autophosphorylation rather than a more conventional pathway involving activation of upstream MAPK kinases (24, 38). Regardless, the need to induce IL-12 is likely a consequence of the fact that without production of this cytokine and the ensuing Th1 response, both host and parasite rapidly succumb from the effects of *Toxoplasma* infection (52, 57).

The physiological necessity of down regulating responses to LPS, and likely other TLR ligands, during *T. gondii* infection is not yet clear. One possibility is that this reflects the need to avoid detrimental hyperimmune responses that would otherwise be triggered by the parasite's own TLR ligands. In this regard, a *Toxoplasma* profilin molecule was recently identified as a ligand for mouse TLR11 (58). It is also possible that inflammatory cytokine suppression allows the parasite to escape the microbicidal effects of molecules such as nitric oxide and 47-kDa GTPases that can be highly effective in parasite destruction (9, 12, 22, 49).

Finally, signaling through MyD88 is reported to be required for maintenance of normal gut homeostasis (46). Nevertheless, it is possible that during oral *Toxoplasma* infection the host intestinal environment is exposed to abnormally high levels of gut flora as a consequence of parasite-induced damage to gut tissue. From this perspective, down-regulating responses to bacterial TLR ligands may allow the parasite to initiate infection without inducing an overwhelming inflammatory response to gut-dwelling bacteria.

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REFERENCES

- Aderem, A., and D. M. Underhill. 1999. Mechanisms of phagocytosis in macrophages. *Annu. Rev. Immunol.* **17**:593–623.
- Akira, S., and T. Kishimoto. 1997. NF-IL6 and NF- κ B in cytokine gene regulation. *Adv. Immunol.* **65**:1–46.
- Bjorkbacka, H., K. A. Fitzgerald, F. huet, X. Li, J. A. Gregory, M. A. Lee, C. M. Ordija, N. E. Dowley, D. T. Golenbock, and M. W. Freeman. 2004. The

- induction of macrophage gene expression by LPS predominantly utilizes MyD88-independent signaling cascades. *Physiol. Genomics* **19**:319–330.
- Bliss, S. K., Y. Zhang, and E. Y. Denkers. 1999. Murine neutrophil stimulation by *Toxoplasma gondii* antigen drives high level production of IFN- γ -independent IL-12. *J. Immunol.* **163**:2081–2088.
- Bondeson, J., K. A. Browne, F. M. Brennan, B. M. Foxwell, and M. Feldmann. 1999. Selective regulation of cytokine induction by adenoviral gene transfer of IkappaBalpha into human macrophages: lipopolysaccharide-induced, but not zymosan-induced, proinflammatory cytokines are inhibited, but IL-10 is nuclear factor-kappaB independent. *J. Immunol.* **162**:2939–2945.
- Bradley, M. N., L. Zhou, and S. T. Smale. 2003. C/EBP β regulation in lipopolysaccharide-stimulated macrophages. *Mol. Cell. Biol.* **23**:4841–4858.
- Brightbill, H. D., S. E. Plevy, R. L. Modlin, and S. T. Smale. 2000. A prominent role for Sp1 during lipopolysaccharide-mediated induction of the IL-10 promoter in macrophages. *J. Immunol.* **164**:1940–1951.
- Butcher, B. A., and E. Y. Denkers. 2002. Mechanism of entry determines ability of *Toxoplasma gondii* to inhibit macrophage proinflammatory cytokine production. *Infect. Immun.* **70**:5216–5224.
- Butcher, B. A., R. I. Greene, S. C. Henry, K. L. Annecharico, J. B. Weinberg, E. Y. Denkers, A. Sher, and G. A. Taylor. 2005. p47 GTPases regulate *Toxoplasma gondii* survival in activated macrophages. *Infect. Immun.* **73**:3278–3286.
- Butcher, B. A., L. Kim, P. F. Johnson, and E. Y. Denkers. 2001. *Toxoplasma gondii* tachyzoites inhibit proinflammatory cytokine induction in infected macrophages by preventing nuclear translocation of the transcription factor NF κ B. *J. Immunol.* **167**:2193–2201.
- Butcher, B. A., L. Kim, A. Panopoulos, S. S. Watowich, P. J. Murray, and E. Y. Denkers. 2005. Cutting edge: IL-10-independent STAT3 activation by *Toxoplasma gondii* mediates suppression of IL-12 and TNF- α in host macrophages. *J. Immunol.* **174**:3148–3152.
- Collazo, C. M., G. S. Yap, G. D. Sempowski, K. C. Lusby, L. Tassarollo, G. F. Vande Woude, A. Sher, and G. A. Taylor. 2001. Inactivation of LRG-47 and IRG-47 reveals a family of interferon- γ -inducible genes with essential, pathogen-specific roles in resistance to infection. *J. Exp. Med.* **194**:181–187.
- Denkers, E. Y., L. Kim, and B. A. Butcher. 2003. In the belly of the beast: subversion of macrophage proinflammatory signaling cascades during *Toxoplasma gondii* infection. *Cell. Microbiol.* **5**:75–83.
- Dubey, J. P. 1998. Advances in the life cycle of *Toxoplasma gondii*. *Int. J. Parasitol.* **28**:1019–1024.
- Fitzgerald, K. A., D. C. Rowe, and D. T. Golenbock. 2004. Endotoxin recognition and signal transduction by the TLR4/MD2-complex. *Microbes Infect.* **6**:1361–1367.
- Foey, A. D., S. L. Parry, L. M. Williams, M. Feldmann, B. M. Foxwell, and F. M. Brennan. 1998. Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF- α : role of the p38 and p42/44 mitogen-activated protein kinases. *J. Immunol.* **160**:920–928.
- Grove, M., and M. Plumb. 1993. C/EBP, NF- κ B and c-Ets family members and transcriptional regulation of the cell-specific and inducible macrophage inflammatory protein 1 α immediate early gene. *Mol. Cell. Biol.* **13**:5276–5289.
- Hampton, R. Y., D. T. Golenbock, M. Penman, M. Krieger, and C. R. H. Raetz. 1991. Recognition and plasma clearance of endotoxin by scavenger receptors. *Nature* **352**:342–352.
- Huang, Y., T. Li, D. C. Sane, and L. Li. 2004. IRAK1 serves as a novel regulator essential for lipopolysaccharide-induced interleukin-10 gene expression. *J. Biol. Chem.* **279**:51697–51703.
- Ishida, Y., T. Kondo, K. Tsuneyama, P. Lu, T. Takayasu, and N. Muaida. 2004. The pathogenic roles of tumor necrosis factor receptor p55 in acetaminophen-induced liver injury in mice. *J. Leukoc. Biol.* **75**:59–67.
- Kambayashi, T., C. O. Jacob, D. Zhou, N. Mazurek, M. Fong, and G. Strassmann. 1995. Cyclic nucleotide phosphodiesterase type IV participates in the regulation of IL-10 and in the subsequent inhibition of TNF- α and IL-6 release by endotoxin-stimulated macrophages. *J. Immunol.* **155**:4909–4916.
- Khan, I. A., J. D. Schwartzman, T. Matsuura, and L. H. Kasper. 1997. A dichotomous role for nitric oxide during acute *Toxoplasma gondii* infection in mice. *Proc. Natl. Acad. Sci. USA* **94**:13955–13960.
- Kim, L., B. A. Butcher, and E. Y. Denkers. 2004. *Toxoplasma gondii* interferes with lipopolysaccharide-induced mitogen-activated protein kinase activation by mechanisms distinct from endotoxin tolerance. *J. Immunol.* **172**:3003–3010.
- Kim, L., L. Del Rio, B. A. Butcher, T. H. Mogensen, S. Paludan, R. A. Flavell, and E. Y. Denkers. 2005. p38 MAPK autophosphorylation drives macrophage IL-12 production during intracellular infection. *J. Immunol.* **174**:4178–4184.
- Lee, W.-S., S.-J. Yoon, T.-K. Yoon, K.-Y. Cha, S.-H. Lee, S. Shimasaki, S. Lee, and K.-A. Lee. 2004. Effects of bone morphogenetic protein-7 (BMP-7) on primordial follicular growth in the mouse ovary. *Mol. Reprod. Dev.* **69**:159–163.
- Luder, C. G. K., M. Aligner, C. Lang, N. Bleicher, and U. Gross. 2003. Reduced expression of the inducible nitric oxide synthase after infection with

- Toxoplasma gondii* facilitates parasite replication in activated murine macrophages. *Int. J. Parasitol.* **33**:833–844.
27. Luder, C. G. K., C. Lang, M. Giraldo-Velasquez, M. Aligner, J. Gerdes, and U. Gross. 2003. *Toxoplasma gondii* inhibits MHC class II expression in neural antigen-presenting cells by down-regulating the class II transactivator CIITA. *J. Neuroimmunol.* **134**:12–24.
 28. Luder, C. G. K., T. Lang, B. Beurle, and U. Gross. 1998. Down-regulation of MHC class II molecules and inability to up-regulate class I molecules in murine macrophages after infection with *Toxoplasma gondii*. *Clin. Exp. Immunol.* **112**:308–316.
 29. Luder, C. G. K., W. Walter, B. Beurle, M. J. Maeurer, and U. Gross. 2001. *Toxoplasma gondii* down-regulates MHC class II gene expression and antigen presentation by murine macrophages via interference with nuclear translocation of STAT1 α . *Eur. J. Immunol.* **31**:1475–1484.
 30. Luft, B., and J. S. Remington. 1992. AIDS commentary: toxoplasmic encephalitis in AIDS. *Clin. Infect. Dis.* **15**:211–222.
 31. Luft, B. J., R. Hafner, A. H. Korzun, C. Lepout, D. Antoniskis, E. M. Bosler, D. D. Bourland, R. Uttamchandani, J. Fuhrer, J. Jacobson, P. Morlat, J. Vilde, and J. S. Remington. 1993. Toxoplasmic encephalitis in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **329**:995–1000.
 32. Ma, X., J. M. Chow, G. Gri, G. Carra, F. Gerosa, S. F. Wolf, R. Dzialo, and G. Trinchieri. 1996. The interleukin-12 p40 promoter is primed by interferon- γ in monocytic cells. *J. Exp. Med.* **183**:147–157.
 33. MacMicking, J., Q. Xie, and C. Nathan. 1997. Nitric oxide and macrophage function. *Annu. Rev. Immunol.* **15**:323–350.
 34. Malhotra, R., and M. I. Bird. 1997. L-selectin-a signaling receptor for lipopolysaccharide. *Chem. Biol.* **4**:543–547.
 35. Malhotra, R., R. Preist, M. R. Foster, and M. I. Bird. 1998. P-selectin binds to bacterial lipopolysaccharide. *Eur. J. Immunol.* **28**:983–988.
 36. Mason, N., J. Aliberti, J. C. Caamano, H. C. Liou, and C. A. Hunter. 2002. Identification of c-Rel-dependent and-independent pathways of IL-12 production during infectious and inflammatory stimuli. *J. Immunol.* **168**:2590–2594.
 37. Mason, N. J., D. Artis, and C. A. Hunter. 2004. New lessons from old pathogens: what parasitic infections have taught us about the role of nuclear factor- κ B in the regulation of immunity. *Immunol. Rev.* **201**:48–56.
 38. Mason, N. J., J. Fiore, T. Kobayashi, K. S. Masek, Y. Choi, and C. A. Hunter. 2004. TRAF6-dependent mitogen-activated protein kinase activation differentially regulates production of interleukin-12 by macrophages in response to *Toxoplasma gondii*. *Infect. Immun.* **72**:5662–5667.
 39. McKee, A. S., F. Dzierzinski, M. Boes, D. S. Roos, and E. J. Pearce. 2004. Functional inactivation of immature dendritic cells by the intracellular parasite *Toxoplasma gondii*. *J. Immunol.* **173**:2632–2640.
 40. Medvedev, A. E., T. Flo, R. R. Ingalls, D. T. Golenbock, G. Teti, S. N. Vogel, and T. Espevik. 1998. Involvement of CD14 and complement receptors CR3 and CR4 in nuclear factor- κ B activation and TNF production induced by lipopolysaccharide and group B streptococcal cell walls. *J. Immunol.* **160**:4535–4542.
 41. Meisel, C., K. Vogt, C. Platzter, F. Randow, C. Liebenthal, and H. D. Volk. 1996. Differential regulation of monocytic tumor necrosis factor- α and interleukin-10 expression. *Eur. J. Immunol.* **26**:1580–1586.
 42. Morrisette, N., E. Gold, and A. Aderem. 1999. The macrophage—a cell for all seasons. *Trends Cell Biol.* **9**:199–201.
 43. Nemeth, Z. H., G. Hasko, and E. S. Vizi. 1998. Pyrrolidine dithiocarbamate augments IL-10, inhibits TNF- α , MIP-1 α , IL-12, and nitric oxide production and protects from the lethal effect of endotoxin. *Shock* **10**:49–53.
 44. Peltoniemi, J., E. K. Broberg, A. Halenius, N. Setälä, J. P. Eralinna, A. A. Salmi, M. Roytta, and V. Hukkanen. 2004. Immunomodulation by roquinimex decreases the expression of IL-23 (p19) mRNA in the brains of herpes simplex virus type 1 infected BALB/c mice. *Clin. Exp. Immunol.* **137**:305–312.
 45. Plevy, S. E., J. H. M. Gemberling, S. Hsu, A. J. Dorner, and S. T. Smale. 1997. Multiple control elements mediate activation of the murine and human interleukin 12 p40 promoters: evidence of functional synergy between C/EBP and Rel proteins. *Mol. Cell. Biol.* **17**:4572–4588.
 46. Rakoff-Nahoum, S., J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov. 2004. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**:229–241.
 47. Remington, J. S., R. McLeod, and G. Desmonts. 1995. Toxoplasmosis, p. 140–267. *In* J. S. Remington and J. O. Klein (ed.), *Infectious diseases of the fetus and newborn infant*. W. B. Saunders Co., Philadelphia, Pa.
 48. Sanjabi, S., A. Hoffmann, H. C. Liou, D. Baltimore, and S. T. Smale. 2000. Selective requirement for c-Rel during IL-12 P40 gene induction in macrophages. *Proc. Natl. Acad. Sci. USA* **97**:12705–12710.
 49. Scharton-Kersten, T., G. Yap, J. Magram, and A. Sher. 1997. Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. *J. Exp. Med.* **185**:1–13.
 50. Shapira, S., O. S. Harb, J. Caamano, and C. A. Hunter. 2004. The NF- κ B signaling pathway: immune evasion and immunoregulation during toxoplasmosis. *Int. J. Parasitol.* **34**:393–400.
 51. Shapira, S. S., K. Speirs, A. Gerstein, J. Caamano, and C. A. Hunter. 2002. Suppression of NF- κ B activation by infection with *Toxoplasma gondii*. *J. Infect. Dis.* **185**:S66–72.
 52. Sher, A., C. Collazzo, C. Scanga, D. Jankovic, G. Yap, and J. Aliberti. 2003. Induction and regulation of IL-12-dependent host resistance to *Toxoplasma gondii*. *Immunol. Res.* **27**:5221–5528.
 53. Takeda, K., and S. Akira. 2005. Toll-like receptors in innate immunity. *Int. Immunol.* **17**:1–14.
 54. Taylor, G. A., C. G. Feng, and A. Sher. 2004. p47 GTPases: regulators of immunity to intracellular pathogens. *Nat. Rev. Immunol.* **4**:100–109.
 55. Van Amersfoort, E. S., T. J. C. Van Berkel, and J. Kuiper. 2003. Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. *Clin. Microbiol. Rev.* **16**:379–414.
 56. Yang, S., Y. Yang, J. Raycraft, H. Zhang, S. Kanan, Y. Guo, Z. Ronai, I. Hellstrom, and K. E. Hellstrom. 2004. Melanoma cells transfected to express CD83 induce antitumor immunity that can be increased by also engaging CD137. *Proc. Natl. Acad. Sci. USA* **101**:4990–4995.
 57. Yap, G., M. Pesin, and A. Sher. 2000. IL-12 is required for the maintenance of IFN- γ production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. *J. Immunol.* **165**:628–631.
 58. Yarovinsky, F., D. Zhang, J. F. Anderson, G. L. Bannenberg, C. N. Serhan, M. S. Hayden, S. Hieny, F. S. Sutterwala, R. A. Flavell, S. Ghosh, and A. Sher. 2005. TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* **308**:1626–1629.