P2X7 Receptor-Mediated Killing of an Intracellular Parasite, *Toxoplasma gondii*, by Human and Murine Macrophages


*J Immunol* 2010; 184:7040-7046; Prepublished online 19 May 2010;
doi: 10.4049/jimmunol.1000012
http://www.jimmunol.org/content/184/12/7040

**Supplementary Material**
http://www.jimmunol.org/content/suppl/2010/05/19/jimmunol.1000012.DC1

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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
P2X7 Receptor-Mediated Killing of an Intracellular Parasite, Toxoplasma gondii, by Human and Murine Macrophages


The P2X7-R is highly expressed on the macrophage cell surface, and activation of infected cells by extracellular ATP has been shown to kill intracellular bacteria and parasites. Furthermore, single nucleotide polymorphisms that decrease receptor function reduce the ability of human macrophages to kill Mycobacterium tuberculosis and are associated with extrapulmonary tuberculosis. In this study, we show that macrophages from people with the 1513C (rs3751143, NM_002562.4:c.1487A>C) loss-of-function P2X7-R single nucleotide polymorphism are less effective in killing intracellular Toxoplasma gondii after exposure to ATP compared with macrophages from people with the 1513A wild-type allele. Supporting a P2X7-R-specific effect on T. gondii, macrophages from P2X7-R knockout mice (P2X7-R−/−) are unable to kill T. gondii as effectively as macrophages from wild-type mice. We show that P2X7-R-mediated T. gondii killing occurs in parallel with host cell apoptosis and is independent of NO production. The Journal of Immunology, 2010, 184: 7040–7046.

The purinergic P2X-R functions as a proinflammatory receptor in cells of the monocyte/macrophage lineage. P2X7-R cell surface membrane expression is upregulated by IFN-γ, and the receptor is activated by extracellular ATP released from a variety of cellular sources, including platelets and damaged cells (1). Activation of monocyte and macrophage P2X7-R has been shown to kill intracellular Mycobacterium (2–11), Chlamydia (12–14), and Leishmania species (15). The P2X7-R gene (P2RX7) is highly polymorphic, and a number of non-synonymous single nucleotide polymorphisms (SNPs) have been described that alter receptor function. The majority of SNPs, including 946G>A (rs28360457, NM_002562.4:c.920G>A, Arg307 to Gin), 1096C>G (rs2230911, NM_002562.4:c.1070C>G, Thr357 to Ser), Gin Arg 1513A>C (rs3751143, NM_002562.4:c.1487A>C, Gin496 to Ala), and 1729T>A (rs1653624, NM_002562.4:c.1703A>T, Ile568 to Asn) confer a loss-of-function phenotype. Significantly, the loss-of-function 1513A>C SNP reduces the in vitro ability of human macrophages to control Mycobacterium tuberculosis (10). Furthermore, inheritance of the 1513A>C SNP has been associated with susceptibility to extrapulmonary tuberculosis in humans (11, 16).

Similar to Mycobacterium, Chlamydia, and Leishmania spp., the apicomplexan parasite, Toxoplasma gondii, is able to infect and survive in cells of the monocyte/macrophage lineage. T. gondii is an obligate intracellular protozoa that infects approximately one-third of humans worldwide (17). Human infection occurs after ingestion of either tissue cysts in raw/undercooked meat or oocysts from infected cat feces, and can be acquired congenitally following primary maternal infection. Although severe disease can occur in the immunocompetent human host, infection is usually asymptomatic or a mild illness characterized by malaise, lymphadenopathy, fever, and headache (17). Considerable morbidity and mortality in immunosuppressed individuals, in particular toxoplasmic encephalitis, is caused by reactivation of chronic infection, and severe fetal abnormalities can occur in association with primary maternal infection (17).

In this study, we describe three immunocompetent people with toxoplasmosis who prompted further investigation of P2X7-R as a factor influencing host response to T. gondii infection. We show, in studies using human macrophages and P2X7-R knockout (P2X7-R−/−) mice, that P2X7-R activation by extracellular ATP kills T. gondii parasites in infected cells. P2X7-R-mediated T. gondii death occurs in parallel with host–cell apoptosis and is independent of NO production.

Materials and Methods
P2X7-R function and genotyping in toxoplasmosis subjects

Subjects from the Nepean Hospital (Penrith, New South Wales, Australia) provided informed consent for study of their PBMCs. The experimental protocol was approved by the Sydney West Area Health Service, the University of Sydney Human Ethics Committees, and the University of Technology, Sydney, Human Research Ethics Committee, with approval code UTS HREC 2004-077A. Peripheral blood was collected, mononuclear cells
were separated, and macrophages were generated and cultured, as described previously (9). Ethidium bromide uptake that was either reduced to approximately half (S1) tachyzoites were added to appropriate wells and left to invade for 2 h at 37°C in 5% CO2. Uninvaded parasites were removed, and then the infected cells were treated with ATP (pH 7.4) for 1 h at 37°C in 5% CO2. The media were carefully removed, and the chamber was discarded. Determination of viability was undertaken using acridine orange and ethidium bromide, as described previously (23–25). Cells and parasites were viewed on an Olympus BX51 fluorescent microscope with excitation filter 470/20 nm. A minimum of 300 cells or intracellular parasites was counted per sample, and the data was repeated in duplicate on at least three separate occasions. Images were taken using an Olympus DP70 digital camera at X1000 magnification.

In vivo parasite burden and NO assay

Eight-week-old male mice were infected by i.p. injection of 500 tachyzoites of T. gondii RH or ME49 strain. Splenic parasite burdens were determined for the individual mice using a modification of the method described previously (26). Briefly, spleens were removed and placed into RPMI 1640 containing 5% FCS. Spleens were weighed and single-cell suspensions were made by passing spleens through a 70-μm sieve. Cells were pelleted at 1500 × g, and then resuspended in 4 ml RPMI 1640 containing 5% FCS. One hundred microliters was added to the first well of a 96-well plate, and splenocytes were serially diluted 1/2 across the plate. Plates were incubated at 37°C in 5% CO2 for 8 d before wells were examined for the presence of parasites. parasite burden was determined as the last well in which a single parasite was visible, and the number of parasites per gram was calculated as follows: (mean of reciprocal titer from each duplicate/weight of homogenized spleen) × 400, where 400 is the reciprocal fraction of the homogenized spleen inoculated into the first sample well. Serum was collected from the same mice and assayed for NO using the Griess assay, as described previously (27).

Annexin V FITC and propidium iodide apoptosis

RAW 264.7 cells were seeded into six-well plates containing drop-in Teflon cups (Savillex, Minnetonka, MN) at a density of 1 × 10⁴ cells/well. T. gondii tachyzoites (1 × 10⁴) were infected into each well overnight, prior to the addition of ATP to activate P2X7 receptors. Annexin V FITC was assessed using a modification of ATP using the annexin V FITC apoptosis detection kit (Calbiochem, Merck, Darmstadt, Germany), according to the manufacturer’s instructions. Annexin V FITC and propidium iodide fluorescence were assessed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).

Results

Description of three immunocompetent subjects with toxoplasmosis and reduced or absent P2X7R function

Subject 1 (S1), a 14-y-old male, presented to the Nepean Hospital with a 2-y history of fatigue, lethargy, and generalized painless lymphadenopathy. There was no resolution of symptoms after multiple courses of antibiotic therapy. T. gondii serology was positive for IgM and negative for IgG. A biopsy of an enlarged left axillary lymph node showed lymphadenitis consistent with toxoplasmosis. Symptoms resolved after treatment with sulfadiazine and pyrimethamine, and convalescent serology was positive for Toxoplasma IgG and negative for IgM. Subject 2 (S2), a 20-y-old female, presented with an enlarged submandibular lymph node 5 wk following a dental extraction. Excision lymph node biopsy and histology were consistent with T. gondii lymphadenitis, and T. gondii serology was positive for IgM and IgG. Repeat serology 2 y later was positive for IgG and negative for IgM. Subject 3 (S3), a 24-y-old pregnant female, had a routine fetal ultrasound scan at 18 wk gestation, which showed borderline fetal cerebral ventriculomegaly. A repeat scan at 22 wk gestation showed prominent cerebral ventriculomegaly. A diagnostic amniocentesis was performed, and PCR for T. gondii DNA was positive.

In all three subjects, there was no evidence of any major underlying immunodeficiency: HIV serology was negative; absolute lymphocyte numbers and lymphocyte subsets were normal; absolute neutrophil counts were normal; and serum Ig levels were normal (Fig. 1A). However, all three people displayed impaired macrophage P2X7R function measured by ATP-induced ethidium bromide uptake that was either reduced to approximately half (S1)
or completely absent (S2 and S3) compared with control people (Fig. 1B). Moreover, P2RX7 genotyping showed S1 was heterozygous for 1513A>C, S2 was heterozygous for both 946G>A and 1096C>G, and S3 was heterozygous for both 946G>A and 1513A>C, loss-of-function SNPs (Fig. 1C).

**ATP-dependent macrophage killing of T. gondii is reduced in people with decreased P2X-R function**

Genotyping of the NCCCTS cohort (Fig. 2A) identified people with the most common loss-of-function polymorphism in P2RX7, the 1513A>C polymorphism (28), as well as those with wild-type P2RX7 or polymorphisms in linkage disequilibrium (1068G>A and 1772G>A) that do not reduce the function of the receptor (28). A reduction in parasite burden was observed following ATP treatment of monocyte-derived macrophages from people with wild-type P2RX7 or with no loss-of-function polymorphisms (Fig. 2B). Conversely, ATP treatment of monocyte-derived macrophages cultured from people who are homozygous for the 1513A>C loss-of-function polymorphism had minimal effect on the number of intracellular T. gondii tachyzoites (Fig. 2B).

**FIGURE 1.** Studied acute toxoplasmosis subjects have normal immune function, but have low P2X-R function due to P2X-R loss-of-function SNPs. A, Hb (g/l), WCC (×10⁹ cells/l), absolute lymphocyte counts (Abs lymph; ×10⁹ cells/l), CD4⁺ lymphocyte counts (CD4; ×10⁶ cells/l), CD8⁺ lymphocyte counts (CD8; ×10⁶ cells/l), serum Igs, and HIV serology for HIV was negative for all subjects. B, Monocyte-derived macrophages from S1–3 and from subjects wild type for known loss-of-function P2X-R SNPs were treated with 1 mM ATP, followed by quantification of ethidium bromide flux through P2X-R-generated pores by time-resolved flow cytometry. P2X-R function of cultured monocyte-derived macrophages from S1–3 was decreased in comparison with three normal control subjects. C, Sequencing of the P2RX7 gene shows the presence of one or more nonsynonymous SNPs in all three toxoplasmosis subjects. °Loss-of-function SNP.

<table>
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<tr>
<th>Subject</th>
<th>Hb</th>
<th>WCC</th>
<th>Abs lymph</th>
<th>CD4</th>
<th>CD8</th>
<th>Serum Ig</th>
<th>HIV</th>
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<td>Neg</td>
</tr>
<tr>
<td>S3</td>
<td>120</td>
<td>7.5</td>
<td>2.3</td>
<td>858</td>
<td>988</td>
<td>Normal</td>
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**ATP-dependent macrophage killing of T. gondii is reduced in P2X-R⁻/⁻ mice**

The number of samples and the quantity of cells from people in the NCCCTS cohort with polymorphisms in their P2X-R were relatively limited. Therefore, P2X-R⁻/⁻ mice on a C57BL/6J background were also used to more definitively assess the ability of the P2X-R to mediate killing of T. gondii tachyzoites by macrophages. C57BL/6J mice possess a proline to leucine polymorphism at aa 451 in the C-terminal tail of the P2X-R (29). This polymorphism has variable effects on P2X-R function (29–34), so we included comparative analyses of BALB/c cells in our investigations because this strain of mouse is known to possess fully functional P2X-R (29–34) and is also known to be more resistant to T. gondii than C57BL/6J mice (35). P2X-R function of macrophages from BALB/c, C57BL/6J, and P2X-R⁻/⁻ mice was determined, confirming 100%, 50%, and zero P2X-R-dependent pore opening, respectively (Supplemental Fig. 1). ATP treatment of macrophages from BALB/c and C57BL/6J mice resulted in a marked reduction in viability of T. gondii RH strain. In contrast, ATP treatment of

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>946G&gt;A</th>
<th>1068G&gt;A</th>
<th>1096C&gt;G</th>
<th>1513A&gt;C</th>
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<tr>
<td>Amino Acid</td>
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<td>Ala-348-Thr</td>
<td>Ser-357-Thr</td>
<td>Glu-496-Ala</td>
</tr>
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<td>S1</td>
<td>G/G</td>
<td>G/A</td>
<td>C/C</td>
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<td>G/A</td>
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<td>C/G</td>
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<tr>
<td>S3</td>
<td>G/A</td>
<td>G/G</td>
<td>C/C</td>
<td>A/C</td>
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</table>
P2X7R-/- murine macrophages produced no significant loss of T. gondii viability (Fig. 3). Very similar results were seen with the relatively avirulent, type 2 ME49 strain of T. gondii; exposure of BALB/c macrophages to ATP reduced parasite viability from 81 ± 4% to 49 ± 12%, in C57BL/6J macrophages, from 83 ± 2% to 49 ± 12%, but, in P2X7R-KO mice, parasite viability remained unchanged at 82 ± 4% (versus 83 ± 3% in nonactivated cells; results are mean ± SE, n = 3).

There were significant differences in the number of parasites recovered from the spleens of ME49-infected mice: on day 12 postinfection, for BALB/c mice, 1693 ± 238 parasites per gram spleen were recovered versus 2353 ± 500 for C57BL/6J mice and 2892 ± 298 for P2X7R-/- mice (results are means ± SE, n = 6, and there were significantly more parasites recovered from the P2X7R-/- mice, p < 0.05, Mann-Whitney nonparametric test).

P2X7R-mediated killing of T. gondii is independent of NO production, but is associated with host cell apoptosis

To test whether killing of T. gondii after P2X7R activation is dependent on NO production, we compared serum NO levels from P2X7R-deficient infected mice, although none of the differences achieved statistical significance; thus, control of T. gondii RH strain via the P2X7R is independent of NO production (Fig. 4).

RAW 264.7 cells express functional P2X7R (Supplemental Fig. 2). Exposure to ATP induced rapid apoptosis of T. gondii-infected RAW 264.7 cells; more than 70% of cells were positive for annexin V by 1 h (Fig. 5A). At 5 h postexposure to ATP, a significant percentage (30%) of apoptotic cells had progressed toward lysis (Fig. 5A).

Similar levels of ATP-induced apoptosis and necrosis were seen in uninfected RAW 264.7 cells (data not shown).

In situ staining of RAW 264.7 cells with acridine orange and ethidium bromide confirmed the annexin V/propidium iodide flow cytometry results. Acridine orange is able to penetrate all cells and stains nucleic acids green, whereas ethidium bromide, which binds to nucleic acids and fluoresces orange, is only able to enter cells once the integrity of the cell membrane is compromised. Thus, live cells have normal nuclear staining with green chromatin apparent in organized nuclear structures, whereas nonviable cells exhibit orange staining of nuclear material and/or condensed or fragmented chromatin (23–25). Cells were viewed and counted on a fluorescence microscope, and the viability of RAW 264.7 cells was assessed according to these criteria (Fig. 5C). Thus, 54% of RAW 264.7 cells were nonviable after 1 h and 86% nonviable within 2 h of exposure to ATP, compared with 4 and 7%, respectively, for untreated cells. Furthermore, at 2 h postexposure to ATP, intensely
FIGURE 3. Deletion of the P2RX7 gene affects the ability of murine macrophages to control T. gondii. T. gondii RH strain tachyzoites (3 x 10^6) were added to 1 x 10^6 macrophages, and parasite viability was assessed by flow cytometry 24 h after addition of 3 mM ATP. Parasite viability was significantly reduced in ATP-treated macrophages from BALB/c (p < 0.0001) and C57BL/6J (p < 0.0001) mice compared with untreated controls. Parasite viability was not significantly reduced after the addition of ATP to macrophages from P2X7R^−/− mice (p > 0.05). Results are the mean ± SE, n = 4. Statistical analysis was performed using a general linear model one-factor ANOVA with Tukey’s post hoc test.

stained, condensed, fragmented areas of chromatin were evident within the nuclei of host cells (Fig. 5B, panel 3). At later time points, microscopic examination revealed extensive cell lysis.

In situ staining with acridine orange and ethidium bromide also showed that loss of viability of intracellular tachyzoites of T. gondii occurred in parallel to host cell apoptosis. Thus, 5 min postexposure to ATP, almost all tachyzoites were stained green with acridine orange (Fig. 5B, panel 1) and, after 1 h, the parasites were still predominantly (73%) acridine orange positive and ethidium bromide negative (Fig. 5B, panel 2; Fig. 5C). However, by 2 h postexposure to ATP, 43% of the parasites were stained orange with ethidium bromide (Fig. 5B, panel 3; Fig. 5C). Meanwhile, tachyzoites exposed to ATP in cell-free media remained 100% viable, even after 2 h (data not shown). This demonstrates that a direct toxic effect of exogenous ATP on T. gondii is unlikely; rather, ATP acts via effects on the host cell.

FIGURE 4. NO production in response to infection with T. gondii is unaffected by deletion of the P2RX7 gene. Mice were infected by i.p. injection with 500 RH T. gondii tachyzoites and euthanized 8 d post-infection. Serum was collected and assayed for NO using the Griess assay. Results are the mean ± SE; BALB/c control/infected, n = 5 of 8; C57BL/6J control/infected, n = 5 of 9; P2X7R^−/− control/infected, n = 5 of 5. There were no statistically significant differences in serum NO levels between strains for mice infected with T. gondii (using a general linear model one-factor ANOVA with Tukey’s post hoc test).

FIGURE 5. T. gondii killing occurs in parallel to host cell apoptosis in murine macrophages. A. Murine macrophages undergo rapid apoptosis after treatment with ATP. RAW 264.7 cells (1 x 10^6) were infected with RH T. gondii tachyzoites (3 x 10^6). After overnight incubation, RAW 264.7 cells were untreated (left panel) or treated for 1 h with 1 mM ATP to activate P2X-R (right panel); apoptosis was quantified by flow cytometry after annexin V FITC/propidium iodide staining 1, 3, and 5 h after addition of 1 mM ATP. Results are mean ± SE, n = 3; green bars are viable cells, yellow are apoptotic cells, and red are late apoptotic or necrotic cells. B and C. 1 x 10^6 RAW 264.7 cells were infected with 3 x 10^6 RH T. gondii tachyzoites for 2 h. Extracellular parasites were washed away, and the monolayer was then treated with 1 mM ATP for 5, 60, or 120 min. parasite viability was determined after staining with acridine orange (viable, green fluorescence) and ethidium bromide (nonviable, red fluorescence) and viewed at x1000 magnification. The number of nonviable tachyzoites was significantly increased at both 60 and 120 min after ATP treatment (p < 0.05). Results are the mean ± SE, n = 4. Statistical analysis was performed using a general linear model two-factor ANOVA with Tukey’s post hoc test, comparing the untreated control with experimental treatments at each time point. Treatment of extracellular parasites with ATP for 120 min did not affect their viability (data not shown).
8, or 16 h of normal in vitro culture (data not shown). The effects on intracellular T. gondii of exposure to ATP could be totally reversed by pretreatment of RAW264.7 cells with oxidized ATP (data not shown), a potent antagonist of the murine P2X7-R (37), further aiding in confirmation of the specificity of the effect.

Discussion

P2X7-R genotyping and relative P2X7-R activity of three acute toxoplasmosis patients from Nepean Hospital (Fig. 1) are consistent with previous studies showing 50% reduction in monocyte P2X7-R function in 1513A>C heterozygotes and complete loss of function for compound heterozygotes (8, 9, 11). To follow up the surprising association between P2X7-R function and acute symptoms in these patients, and to study the effect of polymorphisms at P2RX7 on function in more detail, we accessed cryopreserved cells from NCCCTS individuals of known genotypes/haplotypes (20). We demonstrated that ATP-dependent killing of tachyzoites of T. gondii in vitro was virtually non-existent in people with the 1513A>C polymorphism, but readily observable in macrophages from people with wild-type receptors or in cells from people with polymorphisms that do not cause loss of receptor function (Fig. 2).

The number of cells available from NCCCTS subjects was limited and did not allow us to perform additional, confirmatory studies with antagonists of the P2X7-R. Therefore, we compared parasite killing using macrophages from P2X7-R−/− mice with BALB/c mice and C57BL/6 mice to confirm that reduced parasite killing is P2X7-R specific; cells from the knockout mice were totally unable to affect the viability of T. gondii upon activation with ATP (Fig. 3). These data confirm that ATP-dependent macrophage killing of T. gondii is via P2X7-R and does not involve other P2X or P2Y receptors, which are also activated by extracellular ATP (37). Parasite burden in vivo in BALB/c, C57BL/6, and P2X7-R−/− mice appears to at least partially confirm that the P2X7-R plays a role in controlling T. gondii. Thus, there were differences in the number of parasites recovered from the spleens of ME49-infected mice, in proportions consistent with their relative P2X7-R function.

It perhaps needs to be noted that ATP-induced killing of T. gondii was comparable in BALB/c and C57BL/6 mice even though the C57BL/6 strain is known to possess a proline to leucine polymorphism at Arg451 that reduces ATP-dependent pore formation by 50% (Supplemental Fig. 1). This could be because of the higher concentration of ATP used in the parasite-killing assay versus the pore-opening assay (3 mM versus 1 mM). However, it is also important to recognize that this particular polymorphism has quite variable effects on P2X7-R function depending on the cell type and activity being examined. Thus, for example, it reduces ATP-dependent pore formation (29), impairs cell death in thymocytes (30), inhibits ATP-induced IL-2 production by splenocytes (31), and affects intercellular calcium waves in astrocytes (32). In contrast, this polymorphism has no effect on phospholipase D activation (30), no effect on expression of the P2X7-R (33), and no effect on P2X7-R-mediated calcium influx in bone marrow-derived macrophages or splenocytes (34).

Activation of the P2X7-R initiates a cascade of intracellular events, including activation of NF-κB (38), phospholipase D (39), and metalloproteases (40); release of reactive oxygen and nitrogen intermediates (41); and stimulation of caspases, leading to apoptosis (42). Loss-of-function polymorphisms in the P2X7-R are also recognized (43, 44) to have a negative effect on the activation of the inflammasome, a complex of cytosolic proteins that regulates caspase-1 activation and, therefore, the processing of IL-1β and IL-18 from inactive to active forms. It is known that killing of mycobacteria and Leishmania amazonensis via the P2X7-R is independent of NO (3, 6, 15); rather, it is associated with apoptosis of host cells (2, 3, 7, 8, 10, 11, 15), as is P2X7-R-mediated killing of Chlamydia (12). We confirmed that P2X7-R-mediated killing of T. gondii is also not associated with NO levels (Fig. 4). To test whether T. gondii killing was associated with apoptosis, we studied in vitro parasite death in RAW 264.7 cells after induction of apoptosis with ATP. Both flow cytometry and in situ staining with acridine orange and ethidium bromide enabled us to document that loss of viability of intracellular tachyzoites of T. gondii occurred in parallel to host cell apoptosis (Fig. 5).

The vulnerability of T. gondii to ATP-induced, P2X7-R-mediated killing shares much in common with similar phenomena observed with Mycobacteria (2–11), Chlamydia (12–14), and Leishmania (15) species, not least the association with apoptosis and independence from NO generation. However, there are likely to be differences too; P2X7-R-dependent killing of Mycobacteria and Chlamydia species is actually dependent on phospholipase D, which is associated with both apoptosis and phagosome-lysocome fusion (4–6, 13). This scenario seems less likely for T. gondii as the parasite inhibits phagosome-lysosome fusion after active penetration of the host cell and modification of phagosomal membrane proteins (45–47). A role for caspase-1 and subsequent release of mature IL-1β, which can cause apoptosis in surrounding cells (37), can also be ruled out because RAW 264.7 cells lack the key adaptor protein, apoptosis-associated specklike protein containing a C-terminal caspase-activating recruiting domain, to form the inflammasome (48). Whereas the limitations of the correlative link between host cell apoptosis and parasite killing must be acknowledged, we believe that, on balance, this effect is likely to be P2X7-R specific and does not imply that just any proapoptotic agent will cause killing of this parasite. This is because of the extremely well-demonstrated ability of T. gondii to inhibit apoptosis induced by a remarkable spectrum of extrinsic and intrinsic proapoptotic stimuli in a variety of host cell types (49–55). It is also worth noting that apoptosis induced by CD95 ligation or by H2O2 or necrosis induced by complement does not directly influence the survival of mycobacteria within macrophages (3). Thus, although we have shown that host cell apoptosis is a potential effector mechanism of parasite killing via P2X7-R activation, the precise mechanism underpinning this remains to be definitively determined.

Our observations suggest that inheritance of SNPs that reduce P2X7-R function might cause a defective response to T. gondii infection, higher risk of reactivation in immunocompromised people, and more severe congenital toxoplasmosis. Genotyping P2RX7 in these patient groups and identifying those at risk would allow for more intensive monitoring for reactivation of infection and, if required, suppressive treatment using anti-Toxoplasma therapy.

Disclosures

The authors have no financial conflicts of interest.

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