Human Platelet-mediated Cytotoxicity against
Toxoplasma gondii: Role of Thromboxane

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Summary

Human platelets, in the absence of antibody, are cytotoxic to tachyzoites of Toxoplasma gondii
as determined by vital staining, transmission electron microscopy, and the failure of Toxoplasma
to survive and replicate in mice after in vitro interaction of the organisms with platelets. Platelet
to T. gondii ratios as low as 1:3 were toxic to the organisms with direct cell–cell contact essential
for platelet-mediated cytotoxicity. Adherence of platelets to T. gondii and disruption of surface
membranes and cytoplasmic contents of the organisms were observed ultrastructurally. Reactive
oxygen species were not implicated in the platelet-mediated toxicity. The interaction of T. gondii
with platelets resulted in a marked increase in thromboxane B2 (TXB2) production compared
with that by unstimulated platelets. The cyclooxygenase inhibitors acetylsalicylic acid and indo-
methacin inhibited platelet-mediated cytolytic activity as did the selective TXA2 synthetase
inhibitor dazmegrel, indicating a role for thromboxane in the platelet-induced cytotoxicity. Further,
 toxoplasmicidal activity was retained in the TXA2 synthetase-containing microsomal fractions
of platelets disrupted by freezing and thawing; cytolytic activity was absent in microsome-depleted
platelet supernatant fractions. Both the TXA2-generating platelet microsomal system and a stable
TXA2 analogue induced damage to the cellular membranes of the Toxoplasma as noted by
transmission electron microscopy. These findings suggest that platelets may play a role in the
host defense against Toxoplasma and that release of thromboxane may be important in this
cytolytic process.

Toxoplasma gondii is an obligate intracellular protozoan
that actively invades macrophages without triggering
respiratory burst activity, prevents phagolysosomal fusion,
and replicates intracellularly to the detriment of the host (1–3).
Toxoplasmosis, the disease caused by this intracellular coccidian parasite, is of increasing concern because of its high
incidence in immunocompromised patients (4). Toxoplasmic
encephalitis is the most common cause of focal central nervous
system lesions in patients with AIDS (5). Parasitemia can be
demonstrated in acute infections (6, 7) and in reactivation
toxoplasmosis in immunocompromised patients (8, 9).
Neutrophils (10), monocytes (10, 11), and cytokine (e.g., IFN-
γ)-activated macrophages (11, 12) have cytotoxic activity
against these organisms and contribute toward control of in-
fec tion.

Recent attention has been directed to the cytotoxic activity
of platelets against various target cells. Platelet-induced
antibody-dependent cell-mediated cytotoxicity (ADCC)
against antibody-sensitized sheep (13, 14) and human (15)
erthrocytes, schistosomula of Schistosoma mansoni (16–18),
and microfilariae of Brugia malayi (19) has been demonstrated
in vitro. Platelet-mediated ADCC activity resides preformed
in platelet membranes and may involve phospholipase A2
reaction products (20). Whereas mouse platelet-mediated cy-
totoxicity against erythrocytes is dependent upon C3 and IgG
immunoglobulins (13, 14), human platelet-mediated cytotoxicity
against S. mansoni (16), and B. malayi (19) is dependent
upon IgE. In addition, IFN-γ (18) and C-reactive protein
(17) can each promote platelet killing of schistosomula. Human
platelets are toxic to certain adherent human tumor cell lines
in the absence of antibody (21). This tumoricidal activity is
blocked by inhibitors of arachidonic acid metabolism (21).
Similarly, phospholipase A2 reaction products have been im-
plicated in NK cell–mediated target cell lysis (22–24).

We report here that human platelets are toxic to T. gondii
tachyzoites in the absence of added antibody. Platelet adher-
ence to the surface of the parasites and disruption of the sur-
face membranes and internal architecture of the Toxoplasma
were observed. Further, our results suggest a prominent role
for the cyclooxygenase arachidonate metabolite thromboxane
(TX) A2 in this platelet-mediated toxoplasmicidal activity.

Abbreviations used in this paper: ADCC, antibody-dependent cell-mediated
  cytotoxicity; AO, acridine orange; CGD, chronic granulomatous disease;
  CTA2, carbocyclic thromboxane A2; EB, ethidium bromide; IPA, indirect
  fluorescent antibody; O2, superoxide anion; PD, phosphate-buffered
  saline, Ca2+/Mg2+ free; PPACK, ω-phenylalanyl-L-prolyl-L-arginine chloro-
  methyl ketone; TX, thromboxane.
Materials and Methods

Special Reagents. PG H2 and carbocyclic thromboxane A2 (CTA2) were obtained from Cayman Chemical Co. (Ann Arbor, MI); human thrombin and α-phenylalanyl-1-prolyl-1-sarigenie chloromethyl ketone (PPACK), dihydrolipoic acid from Calbiochem-Behring Corp. (La Jolla, CA); acridine orange (AO) from Allied-Signal Corp. (Morris Township, NJ); acetyl salicylic acid, ethidium bromide (EB), indomethacin, ferricytochrome c, superoxide dismutase (2,500 U/mg), and EDTA, dipotassium salt from Sigma Chemical Co. (St. Louis, MO); and glutaraldehyde from Polysciences, Inc. (Warrington, PA). Dulbecco's PBS (0.14 M NaCl, 2.7 \times 10^{-3} M KCl, 8.1 \times 10^{-3} M Na2HPO4, 1.5 \times 10^{-3} M KH2PO4, 9.0 \times 10^{-4} M CaCl2, and 4.9 \times 10^{-4} M MgCl2, pH 7.2) and Ca2+/Mg2+-free PBS (PD) were obtained from Gibco Laboratories (Grand Island, NY). Indomethacin was dissolved in 0.1 ml Tris buffer, pH 8.1, at a concentration of 10^{-2} M and diluted with PBS. The selective TX synthetase inhibitor, UK-38,485 (dazmegrel), 3-(1H-imidazol-1-ylmethyl)-2-methyl-1H-indole-1-propanoic acid (25) was kindly provided by Dr. P. R. Urquilla (Pfizer, Inc., Groton, CT). Dazmegrel was dissolved in 0.1 N NaOH in PBS at a concentration of 10^{-2} M with dilutions made in PBS. The final pH of the indomethacin and dazmegrel solutions was 7.0.

Toxoplasma gondii. T. gondii RH strain was kindly provided by Dr. C. B. Wilson (University of Washington). The Toxoplasma were maintained by i.p. passage in BALB/c mice as previously described (26). Organisms were harvested by peritoneal lavage using PD, separated from leukocytes by filtration through a 3-μm polycarbonate filter (Nucleopore Corp., Pleasanton, CA), and centrifuged at 1,000 g for 15 min at 4°C. The Toxoplasma were washed twice by sequential resuspension in PD and centrifugation at 1,000 g for 15 min at 4°C. After counting in a hemocytometer, the Toxoplasma were resuspended in PBS. A clinical isolate of T. gondii (ER strain) was isolated from the brain biopsy of an adult male AIDS patient who had encephalitis. The ER strain Toxoplasma were propagated in human embryonic tonsil fibroblasts, which were a generous gift of Dr. Lawrence Corey (Children's Hospital and Medical Center, Seattle, WA). After washing in PD, the ER strain Toxoplasma were resuspended in PBS. Both the RH and ER strains of Toxoplasma were >95% viable when assessed by trypan blue dye exclusion (27).

Human Subjects. Platelets were isolated from the blood of 24 normal human volunteers and 1 patient with chronic granulomatous disease (CGD). In some studies, volunteers ingested 1.3 mg/kg of acetyl salicylic acid 2 h before blood collection to block platelet formation of cyclooxygenase arachidonate products (28). Otherwise, the subjects had not taken acetyl salicylic acid or other nonsteroidal antiinflammatory drugs for 2 wk before the study.

Toxoplasma-specific IgG and IgM Antibody Tests. Each blood donor was negative for Toxoplasma-specific IgG and IgM antibodies when tested with the indirect fluorescent antibody (IFA) technique performed according to the manufacturer's recommendations (Microbiological Research Corp., Bountiful, UT). Briefly, these tests use RH strain Toxoplasma as the antigen substrate that is dried and fixed on microscope slides. After incubation with test serum, the slides are rinsed and either fluorescein-conjugated anti-human IgG (γ chain specific) or fluorescein-conjugated IgM (μ chain specific) is applied. After a second incubation, the slide is again rinsed and examined under a fluorescence microscope for the typical peripheral staining reaction. The lack of fluorescence at a 1:16 serum IgG-IFA titer and at a 1:8 serum IgM-IFA titer are considered negative, indicating the lack of prior infection (either acute or chronic) with T. gondii.

Platelet Isolation. For platelet isolation, venous blood was collected in K2EDTA (0.2 ml of 10% K2EDTA in 10 ml of blood), diluted 1:1 (vol/vol) with PD and centrifuged at 200 g for 15 min at 4°C. The platelet-rich plasma was collected and then centrifuged at 1,000 g for 15 min at 4°C. The resulting platelet pellet was washed twice by sequential resuspension in PD and centrifugation at 1,000 g for 15 min at 4°C. After counting in a hemocytometer, the platelets were resuspended in PBS and used immediately. The preparations contained <0.05% contamination with nucleated cells.

Platelet-Toxoplasma Interactions. Duplicate samples of Toxoplasma (10^7 organisms/ml) were added to platelets and other components of the reaction mixtures at the concentrations indicated in the legends to the figures and tables in poly styrene tubes (12 × 75 mm) in an oscillating water bath at 37°C for 90 min. Duplicate 10-μl samples were taken from each experimental condition after 0-, 60-, 90-min incubations of platelets or disrupted platelet fractions (see below) with T. gondii for assay of viability of the organisms by trypsin blue staining and AO/EB fluorescence microcopy. After 90 min of incubation, the reaction mixtures were centrifuged at 1,000 g for 15 min at 4°C and the supernatants collected and stored at −70°C until RNAs for TxB2, PGE2, and 6-keto-PGF1α were performed.

For studies to assess the effect of antibody on the platelet-Toxoplasma interactions, immune sera were obtained from (a) an asymptomatic individual with an IgG-IFA titer of 1:2,048 and a negative IgM-IFA titer < 1:8 and (b) a patient with acute toxoplasmosis who had an IgG-IFA titer of 1:1,024 and an IgM-IFA titer of 1:512. Normal serum was obtained from an individual who lacked Toxoplasma-specific antibodies (i.e., IgG-IFA < 1:16 and IgM-IFA < 1:8). Fresh normal sera and normal and immune sera that had been heat-inactivated at 56°C for 45 min were used at a 1:10 dilution (final concentration) in the platelet-Toxoplasma interactions.

To assess the in vivo survival of Toxoplasma that had been incubated with platelets in vitro, 1-ml reaction mixtures containing 10^7 T. gondii that had been incubated in the absence or presence of 10^7 platelets for 90 min at 37°C were injected into duplicate pairs of BALB/c mice. Animals were examined on a daily basis for survival. Mice still living 14 d after injection were classified as survivors and sacrificed to assess i.p. infection with Toxoplasma.

In some experiments, disrupted platelet fractions were substituted for intact platelets in the reactions with T. gondii. Platelets (2 × 10^9) in 1 ml were deglycosylated by treatment with 5 U/ml human thrombin for 15 min at 37°C (29) with the reaction terminated by the addition of 10^-4 M PPACK (30). Platelets (6 × 10^9) in 3 ml of PBS were also disrupted by three cycles of freezing at −70°C for 15 min followed by thawing in a 37°C shaking water bath for 15 min over a 90-min period as previously described (14). The entire contents of the frozen and thawed platelets were used in some experiments or comparably disrupted samples were centrifuged at 5,000 g for 15 min at 4°C for removal of cellular debris. The supernatant was centrifuged at 100,000 g for 60 min at 4°C as previously described (31, 32) to obtain a microsome-depleted supernatant and a microsome-containing particulate fraction which was suspended in 3 ml PBS.

In other studies, intact platelets and T. gondii were cocultured in individual chambers separated by a 0.4-μm polycarbonate membrane in a 6-well (24-mm-diam) Transwell plate (Costar Data Packaging Corp., Cambridge, MA). Toxoplasma (2 × 10^6) were added to 2 ml PBS in the bottom chamber and platelets (2 × 10^9) in 2 ml PBS were placed in the upper chamber. The plates were centrifuged at 1,000 g for 30 min at 20°C to facilitate exchange of media between the chambers and then incubated an additional 60 min at 37°C before assay of T. gondii viability.

Assay of T. gondii Viability. Viability of T. gondii was determined
by trypan blue staining (27) and fluorescence microscopy. Differential
fluorescence of viable and nonviable T. gondii was examined by reac-
tion with AO and EB as previously described (33). Each sample
was mixed with an equal volume of an AO (2.5 μg/ml)/EB
(5 μg/ml) solution before placement on a glass slide in preparation
for fluorescence microscopy using an Olympus Vanox microscope
with epilluminator (Olympus Corporation of America, New Hyde
Park, NY). Toxoplasma were counted at 200× magnification using a
micrometer disc (Bausch & Lomb Inc., Rochester, NY) containing
64 grids (total grid area = 2,000 μm²); 10 random fields were
selected for each sample. Viable organisms demonstrated a bright
green nuclear fluorescence, whereas nonviable Toxoplasma were
orange.

Transmission Electron Microscopy. Pellets of 2 × 10⁷ T. gondii in-
cubated with the various components of the reaction mixture (see
legends to figures) were collected by centrifugation, fixed in 2%
glutaraldehyde, and prepared for transmission electron microscopy
as previously described (34). The samples were examined with a
JEOL 100 B electron microscope (JEOL USA, Electron Optics Di-
vision, Medford, MA) at 60 kV.

Superoxide Anion Assay. Superoxide anion (O₂⁻) generation was
measured by the capacity of O₂⁻ to reduce ferricytochrome c to fer-
cytocrome c in a microassay system as previously described (35).
In brief, the 0.2-ml reaction mixture consisted of PBS, 8% albumin,
54.5 μM ferricytochrome c, 10⁷ platelets, and the presence or ab-
sence of 10⁸ T. gondii. The reactions were performed in quadru-
plicate wells of 96-well microtiter plates (Costar Data Packaging
Corp., Cambridge, MA) in the presence or absence of 62.5 μg/ml
superoxide dismutase for 90 min at 37°C. Nanomoles of ferricy-
tocrome c reduced were calculated from the maximal increase
in absorbance measured at 550 nm in a microtiter plate reader (model
309; Bio-Tek Instruments, Inc., Burlington, VT).

RIs. PGE₂ and the respective stable hydrolysis products of
PGI₂ and TXA₂, 6-keto-PGF₁α, and TXB₂, were assayed by RIA.
The PGE₂, 6-keto-PGF₁α, and TXB₂ antisera were produced in
rabbits in our laboratory and their cross-reactivities have been
reported (36, 37). The PGE₂ antiserum at a dilution of 1:5,000
had a sensitivity of 10 pg per 0.1-ml sample, the 6-keto-PGF₁α an-
tiserum at a 1:6,000 dilution had a sensitivity of 10 pg per 0.1-ml
sample, and the TXB₂ antiserum at a dilution of 1:100,000 had
a sensitivity of 1 pg per 0.1-ml sample. Synthetic standards were
the generous gift of Douglas McCarter (Upjohn Co., Kalamazoo,
MI). Labeled tracers ([³H]PGE₂, [³H]6-keto-PGF₁α, and [³H]TXB₂)
were obtained from New England Nuclear (Boston, MA) and un-
labeled synthetic prostanooid standards were obtained from Cayman
Chemical Co. Each assay was performed in duplicate according to
standard protocols.

Statistical Analysis. The data are reported as the mean ± SE
of the combined experiments. Differences were analyzed for
significance (p < 0.05) by Student’s two-tailed t test for independent
means.

Results

Platelet-mediated Cytotoxicity against T. gondii. Incubation of
T. gondii (RH strain) for 90 min at pH 7.0 with intact
human platelets in the absence of serum resulted in a significant
increase in nonviability of the Toxoplasma to 18.9% from a
background of 6.4% under the conditions used in Table 1. The platelet (effector)/Toxoplasma (target) cell ratio was 1:3.
Toxicity as assessed by trypan blue staining and by orange
fluorescence of the organisms upon vital staining with AO/EB
increased progressively with higher ratios of platelets to Toxo-
plasma up to 84.7% nonviability of the organisms at a 100:1
ratio (Table 1). Human platelets had a similar cytotoxic effect
against a T. gondii strain that had been isolated from a patient
with AIDS; 60.3, 37.0, and 18.9% of these organisms were

Table 1. Toxoplasma-cidal Activity of Human Platelets

| Supplements          | Nonviable T. gondii |
|----------------------|---------------------|
|                      | 0 min   | 60 min | 90 min | p     |
| Toxo (RH)            | 3.7 ± 0.8 (8)* | 6.3 ± 1.5 (8) | 6.4 ± 1.5 (8) | <0.001 |
| Platelets + Toxo (RH) | (1:3)   | 4.0 ± 0.8 (8) | 12.5 ± 1.5 (8) | <0.02  |
|                      | (1:1)   | 3.2 ± 1.5 (3) | 26.4 ± 2.6 (3) | <0.001 |
|                      | (10:1)  | 3.1 ± 1.8 (3) | 34.6 ± 2.9 (3) | <0.001 |
|                      | (100:1) | 3.6 ± 2.0 (3) | 65.6 ± 3.8 (3) | <0.001 |
| Toxo (ER)            | 3.6 ± 0.3 (3) | 4.1 ± 0.9 (3) | 7.5 ± 1.5 (3) | <0.001 |
| Platelets + Toxo (ER) | (1:3)   | 5.3 ± 1.2 (3) | 16.6 ± 2.6 (3) | <0.02  |
|                      | (1:1)   | 5.4 ± 1.1 (3) | 29.1 ± 5.2 (3) | <0.01  |
|                      | (10:1)  | 3.8 ± 1.6 (3) | 42.9 ± 1.3 (3) | <0.001 |

The reaction mixture contained 2 × 10⁷ T. gondii (Toxo; RH strain [RH] or clinical isolate ER strain [ER]) alone or with 0.67 × 10⁹ to 2 ×
10⁹ human platelets as indicated in a total volume of 2 ml PBS. Viability of T. gondii was assessed by trypan blue staining and confirmed by AO/EB
fluorescence microscopy after 0-, 60-, or 90-min incubations at 37°C.

* Mean ± SE of experiments (n). Probability (p) values for the difference from T. gondii (RH strain or clinical isolate) alone are shown where signif-
ican of significant (p < 0.05).
nonviable after 90 min of incubation of platelets with Toxoplasma at ratios of 10:1, 1:1, and 1:3, respectively (Table 1).

Platelet-mediated toxoplasmacidal activity was not affected by the presence of either heat-inactivated (Table 2) or fresh (data not shown) normal serum that was negative for Toxoplasma-specific IgG and IgM antibodies. Further, heat-inactivated immune sera that contained Toxoplasma-specific IgG antibodies in either the absence or presence of Toxoplasma-specific IgM antibodies did not alter platelet-induced cytotoxicity against T. gondii (Table 2). Fresh immune serum was not used since, as reported by other investigators, Toxoplasma-specific antibody in the presence of complement has a cytotoxic effect against the organisms (38-41).

To assess whether the demonstrated in vitro cytotoxic effect of platelets against Toxoplasma corresponded to decreased viability of the organisms in vivo, T. gondii alone and platelet-T. gondii (10:1 ratio) reaction mixtures (each containing 10^7 organisms) after in vitro incubation for 90 min at 37°C were injected intraperitoneally into BALB/c mice; the animals were followed for survival and recovery of viable organisms over a 2-wk study period. All control animals injected with T. gondii alone (n = 8) died within 5 d after injection. In contrast, 100% of the animals receiving the platelet-T. gondii suspensions (n = 8) survived the 14-d study period. Toxoplasma were recovered at necropsy from the peritoneal fluid of each of the mice that had received T. gondii alone but were absent in the peritoneal fluid of the survivors that were sacrificed on day 14.

Direct cell–cell contact was essential for platelet-mediated toxoplasmacidal activity. Separation of platelets from Toxoplasma by a 0.4-μm polycarbonate membrane barrier in a tissue culture well blocked in vitro platelet-mediated toxoplasmacidal activity (Fig. 1).

Platelet-mediated cytotoxicity against T. gondii was confirmed by transmission electron microscopy. Greater than 90% of Toxoplasma incubated in PBS for 90 min exhibited the usual morphology of viable tachyzoites (42). The crescent shaped T. gondii have a continuous outer surface membrane (Fig. 2).

Table 2. Effect of Toxoplasma-specific Antibodies on Platelet-mediated Toxoplasmacidal Activity

| Supplements                  | 0 min | 90 min |  
|------------------------------|-------|--------|  
| Toxo                         | 3.0 ± 1.1 | 6.9 ± 0.6 |  
| + normal serum (IgG-/IgM-)   | 4.7 ± 1.8 | 6.9 ± 0.1 |  
| + immune serum (IgG+/IgM-)   | 4.4 ± 1.4 | 10.8 ± 2.4 |  
| + immune serum (IgG+/IgM+)   | 5.5 ± 1.3 | 10.6 ± 1.4 |  
| Platelets + Toxo             | 4.5 ± 1.6 | 44.5 ± 7.6 | <0.01  
| + normal serum (IgG-/IgM-)   | 5.7 ± 2.3 | 45.5 ± 2.0 | <0.001  
| + immune serum (IgG+/IgM-)   | 4.9 ± 1.2 | 42.4 ± 3.0 | <0.001  
| + immune serum (IgG+/IgM+)   | 4.9 ± 1.3 | 44.9 ± 8.2 | <0.001  

The reaction mixtures contained 2 × 10^7 T. gondii (Toxo; RH strain) in the absence or presence of 2 × 10^7 platelets in 2 ml PBS. In some studies as described in Materials and Methods, normal serum (IgG-IFA <1:16/IgM-IFA <1:8 [IgG-/IgM-]) or immune serum (IgG-IFA = 1:2,048/IgM-IFA <1:8 [IgG+/IgM-]) or IgG-IFA = 1:1,024/IgM-IFA = 1:512 [IgG+/IgM+] that had been previously heat inactivated at 56°C for 45 min was included at a 1:10 dilution (final concentration). The reaction mixtures were incubated for 90 min at 37°C with parasite viability determined as described in Table 1. The data represent the mean ± SE of three experiments. Probability values for the difference from T. gondii alone are shown where significant (p < 0.05).
beneath which is located an inner membranous layer that is interrupted at the anterior and posterior ends of the organisms. The nucleus and intracellular organelles such as Golgi apparatus, mitochondria, and endoplasmic reticulum of the *T. gondii* appeared normal in the control Toxoplasma (Fig. 2 A). The usual array of cytoplasmic organelles is seen including Golgi apparatus (G), mitochondria (M), and nucleus (N). The surface membrane of the Toxoplasma consists of a characteristic double structure with inner and outer membrane units (arrowheads). Bar, 1 μm. × 24,000. (B) Purified human platelets were incubated in buffer for 90 min at 37°C. Pseudopodal surface projections are evident; the platelets (P) contain both α granules (arrowheads) and dense granules (arrows) in their cytoplasm; platelet aggregation was not evident. Bar, 1 μm. × 12,000.

When platelets were incubated with *T. gondii* at a ratio of 10:1 for 30 min, circumferential attachment of the platelets by surface projections to the outer membrane of the parasites was observed (Fig. 3, A and B). The parasites exhibited marked cytotoxic changes as a consequence of this interaction with human platelets for 90 min (Fig. 4, A and B). Swelling and disruption of the parasite surface membrane structure was noted (Fig. 4, A and B); the cytoplasm of the Toxoplasma had numerous surface projections, and aggregation was not evident (Fig. 2 B).
Figure 3. Adherence of platelets to Toxoplasma. (A) Human platelets were incubated with Toxoplasma in a ratio of 10:1 for 30 min as described in Table 1. Close apposition of platelet (P) pseudopodal projections (arrowheads) to the surface of the Toxoplasma (TOXO) is seen. Bar, 1 μm. ×40,000. (B) Platelet (P) adherence (arrowheads) to the surface membrane of the Toxoplasma was often extensive Bar, 1 μm. ×35,000.
Figure 4. Platelet-induced *Toxoplasma* cytotoxicity. (A) Platelets and *Toxoplasma* (10:1 ratio) were incubated for 90 min as described in Table 1. Platelet (P) aggregation and degranulation are evident with attachment (arrowheads) of platelet surface projections to the surface of the *Toxoplasma* (TOXO). Release of cytoplasmic contents (arrows) through the inner parasite membrane is associated with prominent bulging of the outer parasite membrane. Bar, 1 μm. ×30,000. (B) Additional cytotoxic changes noted in the *Toxoplasma* (TOXO) after their interaction with human platelets (P) include disruption of the surface membrane (arrowheads), swelling of the membrane surrounding the nucleus (N), and intracytoplasmic vacuolization with disappearance of intracellular organelles (mitochondria, Golgi apparatus, and endoplasmic reticulum). Bar, 1 μm. ×28,000.
became vacuolated with loss of organelles (Fig. 4B). Other cytotoxic changes noted in the T. gondii were dilation of their nuclear envelope membranes and disruption of their inner surface membranes; platelet aggregation was prominent in the platelet-T. gondii reaction mixtures.

Lack of Role of Reactive Oxygen Species in Cytotoxicity. Studies were conducted to determine the biochemical mechanism(s) of platelet cytotoxicity against T. gondii. Production of small amounts of chemiluminescence (44, 45) and O2 (46) by human platelets has been reported, and the possible contribution of platelet generation of reactive oxygen species in killing of Toxoplasma was examined. Human platelets, however, failed to reduce ferricytochrome c after incubation with T. gondii (10:1 ratio) for 90 min at 37°C (n = 3) indicating a lack of O2 formation during this interaction. In addition, platelets from a CGD patient effectively killed Toxoplasma at platelet/T. gondii ratios from 1:1 to 100:1 at 60 and 90 min of incubation (Fig. 5).

Release of Cyclooxygenase Arachidonate Products during Platelet-T. gondii Interaction. The possible role of cyclooxygenase products of arachidonic acid metabolism in the mediation of platelet cytotoxicity against T. gondii was studied. As seen in Fig. 6, human platelets incubated in buffer alone for 90 min released 34.8 pg TXB2 and 19.4 pg PGE2 per 10^6 platelets. PGE2 (73.4 pg/10^6 organisms) was the predominant cyclooxygenase arachidonate product released by T. gondii incubated in buffer alone for 90 min. When platelets were incubated with Toxoplasma (1:1 ratio), TXB2 production was augmented 3.9-fold (to a concentration of 4 x 10^-9 M) compared to TXB2 release by platelets alone (p < 0.02); PGE2 release was not significantly altered from that of T. gondii alone. Dazmegrel, a potent inhibitor of TXA2 synthetase, has no significant activity against either cyclooxygenase or PGI2 synthetase enzymes (25). Dazmegrel (10^-5 M) inhibited TXB2 release by 85.1% and stimulated PGE2 release by 65.2% in the platelet-T. gondii reaction mixtures. Platelets were also isolated from individuals who had taken acetylsalicylic acid (1.3 mg/kg) 2 h before blood collection for in vivo inhibition of cyclooxygenase activity (28). After interaction with Toxoplasma, these platelets released < 7 pg of either TXB2 or PGE2 per 0.1-ml sample containing 10^6 platelets and/or Toxoplasma (data not shown).
The reaction mixtures contained 2 x 10⁷ T. gondii (Toxo; RH strain) in the absence or presence of 2 x 10⁸ platelets in 2 ml PBS. In some experiments, indomethacin (Indo) or dazmegrel (Daz) at the indicated concentrations were included in the reaction mixtures. Platelets were also obtained from individuals ingesting 1.3 mg/kg of acetylsalicylic acid (ASA) 2 h before blood collection. The supernatants were assayed in 0.1-ml aliquots for TXB₂ by RIA. The reaction mixtures were incubated for 90 min at 37°C with parasite viability assessed as described in Table 1. Mean ± SE of three experiments. Probability values for the difference from T. gondii alone (p₁) and platelets plus T. gondii (p₂) are shown where significant (p < 0.05).

Role of TX Release in Platelet-induced Toxicity. Inhibition of the cyclooxygenase pathway of arachidonic acid metabolism blocked platelet-mediated parasite killing (Table 3). Platelet-mediated toxoplasmacidal activity was inhibited in a concentration-dependent manner by indomethacin and dazmegrel with 85.2 and 82.1% inhibition, respectively, at a 10⁻⁵ M concentration of each inhibitor alone. A direct correlation between degree of inhibition of TX release and Cytotoxicity and Release of TX

Table 3. Effect of Cyclooxygenase and Thromboxane Synthetase Inhibitors on Platelet Toxoplasmacidal Activity and TX Release

| Supplements          | Nonviable T. gondii | TXB₂          |
|----------------------|---------------------|---------------|
|                      | %                   | pg/0.1 ml     | p    |
| Toxo                 | 5.3 ± 1.6*          | 4.3 ± 2.8*    | <0.01|
| Platelets + Toxo     | 60.3 ± 5.3          | 184.7 ± 36.6  | <0.01|
| + Indo (10⁻⁵ M)      | 8.9 ± 2.1           | 14.0 ± 5.6    | <0.02|
| + Indo (10⁻⁶ M)      | 8.0 ± 1.2           | 18.8 ± 7.6    | <0.02|
| + Indo (10⁻⁷ M)      | 22.5 ± 2.9          | 39.7 ± 13.9   | <0.05|
| + Indo (10⁻⁸ M)      | 46.0 ± 0.8          | 159.0 ± 38.4  | <0.02|
| + Indo (10⁻⁹ M)      | 23.7                | (13.9)        |      |
| + Daz (10⁻⁵ M)       | 10.8 ± 2.1          | 16.0 ± 4.0    | <0.02|
| + Daz (10⁻⁶ M)       | 12.1 ± 2.5          | 22.7 ± 9.0    | <0.02|
| + Daz (10⁻⁷ M)       | 44.2 ± 3.3          | 112.0 ± 16.1  | <0.01|
| + Daz (10⁻⁸ M)       | 26.7                | (39.4)        |      |
| Platelets (ASA)      | 8.5 ± 2.7           | 0.3 ± 0.3     | <0.01|
| + Toxo               | (85.9)              | (99.8)        |      |

The reaction mixtures contained 2 x 10⁷ T. gondii (Toxo; RH strain) in the absence or presence of 2 x 10⁸ platelets in 2 ml PBS. In some experiments, indomethacin (Indo) or dazmegrel (Daz) at the indicated concentrations were included in the reaction mixtures. Platelets were also obtained from individuals ingesting 1.3 mg/kg of acetylsalicylic acid (ASA) 2 h before blood collection. The supernatants were assayed in 0.1-ml aliquots for TXB₂ by RIA. The reaction mixtures were incubated for 90 min at 37°C with parasite viability assessed as described in Table 1.

* Mean ± SE of three experiments. Probability values for the difference from T. gondii alone (p₁) and platelets plus T. gondii (p₂) are shown where significant (p < 0.05).

† Percent inhibition.

Table 4. Interaction of T. gondii with Human Platelet Fractions: Cytotoxicity and Release of TX

| Supplements          | Nonviable T. gondii | TXB₂          |
|----------------------|---------------------|---------------|
|                      | %                   | pg/0.1 ml     | p    |
| Toxo                 | 8.6 ± 0.9 (5)*      | 10.4 ± 2.5 (5)| <0.01|
| + intact platelets   | 50.0 ± 12.5 (4)     | 244.4 ± 122.0 (4)| <0.01|
| + thrombin-stimulated platelets | 39.1 ± 4.8 (3) | 197.3 ± 68.1 (3) | <0.01|
| + freeze-thawed platelets: | 30.4 ± 2.7 (3) | <0.001 | 130.7 ± 11.6 (3) | <0.001|
| entire contents      | 100,000-g pellet    | 214.3 ± 65.6 (3) | <0.01|
| 100,000-g supernatant | 10.1 ± 0.5 (3)   | 19.3 ± 4.1 (3)  |      |

The reaction mixtures contained 2 x 10⁷ T. gondii (Toxo; RH strain) in the absence or presence of 2 x 10⁸ platelets in 2 ml PBS. In some experiments, indomethacin (Indo) or dazmegrel (Daz) at the indicated concentrations were included in the reaction mixtures. Platelets were also obtained from individuals ingesting 1.3 mg/kg of acetylsalicylic acid (ASA) 2 h before blood collection. The supernatants were assayed in 0.1-ml aliquots for TXB₂ by RIA. The reaction mixtures were incubated for 90 min at 37°C with parasite viability assessed as described in Table 1.

* Mean ± SE of experiments (n). Probability (p) values for the difference from T. gondii alone are shown where significant (p < 0.05).
Figure 8. Effect of CTA₂ and platelet microsomes on T. gondii morphology. Toxoplasma (TOXO) were incubated in buffer for 90 min at 37°C with either (A and B) 2.9 × 10^{-5} M CTA₂ as described in Fig. 7 or (C) the 100,000 g pellet (microsomes) obtained from 2 × 10⁸ platelets that had been disrupted by freezing and thawing as described in Table 4. As seen in A, CTA₂ caused marked vesiculization of the surface membrane (arrows) and dilation of the nuclear envelope (arrowheads) of the Toxoplasma. Lysis of Toxoplasma by the platelet microsome-containing particulate fraction is observed in C. Extensive swelling (arrowheads) and disruption of surface membranes with loss of intracytoplasmic contents are noted. Surface membrane vesiculization (arrows) is also evident. Bars, 1 μm. (A) × 30,000; (B) × 42,000; (C) × 40,000.
cytotoxicity induced by either indomethacin or dazmegrel was observed (Table 3). Further, platelets obtained from individuals who had ingested acetylsalicylic acid before blood collection failed to either exert a significant cytotoxic effect or release TXB2 after interaction with Toxoplasma (Table 3).

Since TXA2 is unstable with a short half-life of \( \sim 30 \) s at pH 7.4 and 37°C (47), the stable TX analogue CTA2 (48) and a platelet microosomal TXA2-generating system were examined for toxoplasmacidal activity. Incubation of Toxoplasma with \( 2.9 \times 10^{-7} \) M CTA2 for 90 min resulted in killing of 22.7% of the organisms; cytotoxicity increased to 67.4% at \( 2.9 \times 10^{-5} \) M CTA2 (Fig. 7). Ultrastructural studies (Fig. 8, A and B) showed that CTA2 (\( 2.9 \times 10^{-5} \) M) induced vesicle formation in the surface membranes of the Toxoplasma and dilation of the perinuclear membranes of the organisms.

The entire contents of platelets disrupted either by treatment with 5 U/ml thrombin for 15 min or by three cycles of freezing and thawing over a 90-min period which completely releases the platelet cytoplasmic granule contents as previously shown (14) caused a significant reduction in the viability of the Toxoplasma and the respective release of 197.3 and 130.7 pg of TXB2 per 0.1-ml sample (Table 4). The platelets that had been disrupted by freezing and thawing were centrifuged at 5,000 g for 15 min. Additional centrifugation of the supernatant at 100,000 g for 60 min produced a microsome-containing particulate fraction and a microsomedepleted supernatant as previously demonstrated (31). TX synthetase, which is located in platelet microsomes (31, 32), converts the unstable cyclic endoperoxides PGG2 and PGH2 to TXA2 (31, 47). The presence of TXA2 synthetase in the microsome preparations was confirmed by the recovery of 398.0 ± 166.3 pg TXB2 per 0.1-ml sample of the 100,000-g pellet after incubation with \( 10^{-5} \) M PGH2 for 90 min at 37°C (n = 3). The microsome-containing 100,000-g pellet induced a significant increase in nonviability of the organisms. Reaction mixtures containing the platelet 100,000-g particulate fraction and T. gondii released 20.6-fold greater amounts of TXB2 than did T. gondii alone (Table 4). In contrast, the 100,000-g supernatant was ineffective in killing the Toxoplasma and did not induce a significant increase in TXB2 release compared with reaction mixtures with T. gondii alone (Table 4). The toxoplasmacidal activity of the platelet microsomal fractions was confirmed by transmission electron microscopy. T. gondii, after incubation with platelet microsomes (Fig. 8 C), exhibited such cytotoxic changes as swelling of their surface membranes and disruption of their intracellular organelles.

Discussion

These studies demonstrate that human platelets exert potent cytotoxic activity against tachyzoites (i.e., the proliferating form) of the pathogenic protozoan, T. gondii. Platelet toxoplasmacidal activity, like platelet tumoricidal activity (21), occurs in the absence of added antibody. This is in contrast to platelet-mediated ADCC against erythrocytes (13, 14) and infective forms of S. mansoni (16) and B. malayi (19) which require either IgG or IgE antibody for cytotoxicity. Further, heat-inactivated sera containing Toxoplasma-specific IgG antibodies in the absence or presence of Toxoplasma-specific IgM antibodies had no effect on platelet-mediated toxoplasmacidal activity. Platelet-mediated killing of Toxoplasma occurs at platelet/T. gondii ratios as low as 1:3 with toxicity increasing with higher ratios of platelets to organisms over a 90-min period. Prior studies assayed platelet-mediated tumor cell cytotoxicity after a 48-h incubation period (21). Human platelet-induced killing of T. gondii appears to require close contact between effector and target cells since separation of platelets from Toxoplasma by a membrane filter abrogated cytotoxicity. Ultrastructural studies demonstrated initial adherence of platelet pseudopodal projections to the surface of the parasites which was followed by swelling of the outer parasite membrane and intracellular cytolysis of the T. gondii. Toxoplasma after their interaction with platelets in vitro were unable to infect mice in vivo.

Destruction of T. gondii by human platelets was not mediated by the release of toxic oxygen species by the platelets since O2 generation by platelets after interaction with Toxoplasma was not observed and CGD platelets were cytotoxic to the organisms. In addition, platelet granule contents were not implicated in the cytotoxic process since the microsomedepleted supernatant fractions containing granule material obtained from platelets disrupted by freezing and thawing lacked toxoplasmacidal activity. Slezak et al. (14) were similarly unable to demonstrate a role for either platelet-derived oxygen radicals or granule components in platelet-mediated ADCC against sheep erythrocytes. Our data suggest that TX generation may be important in the mediation of platelet killing of Toxoplasma. Evidence in support of this role for TX is as follows.

There was a 3.9-fold increase in TXB2 release by platelets incubated with T. gondii (1:1 ratio) compared with platelets incubated in buffer alone. Inhibition of platelet cyclooxygenase either in vitro by indomethacin or in vivo by acetylsalicylic acid inhibited platelet-mediated parasite killing and release of TXB2. Further, the selective TX synthetase inhibitor dazmegrel abrogated in vitro platelet-mediated killing of Toxoplasma. The selectivity of the inhibitory effect of dazmegrel on TX synthetase vs. cyclooxygenase enzyme activity was demonstrated by the inhibitory effect of dazmegrel on TXB2 release in contrast to its stimulatory effect on PGE2 release by the platelet-T. gondii reaction mixtures. Although T. gondii readily incorporate exogenously added arachidonic acid, the Toxoplasma do not convert arachidonate into lipoxygenase products (26). Generation of PGE2 in the platelet-T. gondii preparations was presumably from the organisms since T. gondii incubated in buffer alone for 90 min released 73.4 pg PGE2 per 0.1-ml sample containing 106 Toxoplasma. Whereas other parasites such as Taenia taeniformis (49, 50) and S. mansoni (51), as well as gram-negative and gram-positive bacteria (52), release PGE2 and other eicosanoids, formation of cyclooxygenase arachidonate products by T. gondii has not been described previously to our knowledge.

Additional evidence for TXA2 in the mediation of platelet-induced cytotoxicity against T. gondii was provided
by studies examining the cytotoxic effect of the TX analogue CTA2 and microsome-containing fractions of disrupted platelets. Although CTA2 lacks the platelet aggregatory activity of TXA2, this stable TX analogue does exhibit the potent vasoconstrictor activity of biologically generated TXA2 (53, 54). Prior studies in cats have demonstrated that CTA2 infusion damages myocardial cell membranes with release of myocardial creatine kinase and large granule hydrolyase activities (54). We found that CTA2 and platelet microsomes capable of releasing TX produced extensive surface and perinuclear membrane damage in the Toxoplasma and induced significant nonviability of the organisms. Since T. gondii form PGE2, it is possible that the organisms provide the cyclic endoperoxide precursor (PGG2/PGH2) required by TXA2 synthetase present in the platelet microsomes to generate TX.

CTA2-induced Toxoplasma cytotoxicity was observed beginning at a concentration of 2.9 × 10⁻⁷ M which is comparable to the 2 × 10⁻⁷ M CTA2 concentration that stimulates the release of lysosomal hydrodases from large granule fractions of liver homogenate (53). In the platelet-T. gondii (1:1 ratio) reaction mixtures, generation of 4 × 10⁻⁹ M TX (Fig. 6) was associated with significant parasite cytotoxicity (Table 1). The greater reactivity of TX in comparison to CTA2 in induction of Toxoplasma cytotoxicity may result from a unique structural feature of TXA2. TXA2 has an acetal carbon atom that binds two oxygen molecules in an extremely strained bicyclic structure that is susceptible to attack by nucleophiles (47), whereas CTA2 is lacking in this structure (53).

TXA2 release by platelets and other inflammatory cells may have additional important effects on the immune response to Toxoplasma besides a direct cytolytic effect. As reported by Tripp et al., (55), TXA2 synthesis is preferentially conserved in Listeria monocytogenes-infected murine peritoneal macrophages. In their studies, 100% of Listeria-infected mice died when treated with indomethacin, in contrast to no deaths of animals in the absence of cyclooxygenase blockade (56). Further, indomethacin-induced dissemination of Listeria was reversed by administration of a stable TXA2 analogue suggesting that TXA2-induced vasoconstriction may help localize the organisms to the initial site of infection (56).

Our studies demonstrate a novel cytotoxic role for human platelets. Platelet adherence to the surface of T. gondii may occur during parasitemia or at inflammatory sites of toxoplasmal infection. We are currently investigating what factors promote this contact. Such interaction may result in platelet aggregation and release of TXA2 and other products which induce cytotoxic damage to the organisms, thus contributing to the host defense against this pathogen.

We thank Dr. P. R. Urquilla (Pfizer, Inc.) for the generous gift of dazmegrel; Dr. Tom Carty, (Pfizer, Inc.) for helpful discussions; Gertrude Chiang, Dong Nguyen, Margot McCready, and Jean Reding for skilled technical assistance; and Rachel Norris for typing this manuscript.

This work was supported by National Institutes of Health grant AI23713.

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Received for publication 14 May 1990 and in revised form 2 August 1990.

References

1. Jones, T.C., and J.G. Hirsch. 1972. The interaction between Toxoplasma gondii and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. J. Exp. Med. 136:1173.
2. Wilson, C.B., V. Tsai, and J.S. Remington. 1980. Failure to trigger the oxidative metabolic burst by normal macrophages. Possible mechanism for survival of intracellular pathogens. J. Exp. Med. 151:328.
3. Murray, H.W. 1986. Cellular resistance to protozoal infection. Annu. Rev. Med. 37:61.
4. McCabe, R.E., and J.S. Remington. 1990. Toxoplasma gondii. In Principles and Practice of Infectious Diseases. G.L. Mandell, R.G. Douglas, Jr., and J.E. Bennett, editors. Churchill Livingstone, New York. 2090–2103.
5. Luft, B.J., and J.S. Remington. 1988. AIDS commentary. Toxoplasmic encephalitis. J. Infect. Dis. 157:1.
6. Kayhoe, D.E., L. Jacobs, H.K. Beye, and N.B. McCullough. 1995. Acquired toxoplasmosis. Observations on two parasitologically proved cases treated with pyrimethamine and triple sulfonamides. N. Engl. J. Med. 257:1247.
7. Frenkel, J.K., R.W. Weber, and M.N. Lunde. 1960. Acute toxoplasmosis. Effective treatment with pyrimethamine, sulfadiazine, leucovorin calcium, and yeast. JAMA. (J. Am. Med. Assoc). 173:1471.
8. Shepp, D.H., R.C. Hackman, F.K. Conley, J.B. Anderson, and J.D. Meyers. 1985. Toxoplasma gondii reactivation identified by detection of parasitemia in tissue culture. Ann. Intern. Med. 103:218.
9. Hofflin, J.M., and J.S. Remington. 1985. Tissue culture isolation of Toxoplasma from blood of a patient with AIDS. Arch. Intern. Med. 145:925.
10. Wilson, C.B., and J.S. Remington. 1979. Activity of human blood leukocytes against Toxoplasma gondii. J. Infect. Dis. 140:890.
11. Murray, H.W., B.Y. Rubin, S.M. Carrero, A.M. Harris, and E.A. Jaffee. 1985. Human mononuclear phagocyte antiprotozoal mechanisms: oxygen-dependent vs. oxygen-independent activity against intracellular Toxoplasma gondii. J. Immunol. 134:1982.

12. Suzuki, Y., M.A. Orellana, R.D. Schreiber, and J.S. Remington. 1988. Interferon-γ: the major mediator of resistance against Toxoplasma gondii. Science (Wash. DC). 240:516.

13. Soper, W.D., S.P. Bartlett, and H.J. Winn. 1982. Lysis of antibody-coated cells by platelets. J. Exp. Med. 156:1210.

14. Slezak, S., D.E. Symer, and H.S. Shin. 1987. Platelet-mediated cytotoxicity. Role of antibody and C3, and localization of the cytotoxic system in membranes. J. Exp. Med. 166:489.

15. Lovechik, J., and R. Hong. 1974. Characterization of effectors and target cell populations in antibody-dependent cell-mediated cytosis. Fed. Proc. 33:780. (Abstr.)

16. Joseph, M., C. Auriault, A. Capron, H. Vornig, and P. Viens. 1983. A new function for platelets: IgE-dependent killing of schistosomes. Nature (London). 303:810.

17. Bort, D., M. Joseph, M. Pontet, H. Vornig, D. Deslée, and A. Capron. 1986. Rat resistance to schistosomiasis: platelet-mediated cytotoxicity induced by C-reactive protein. Science (Wash. DC). 231:153.

18. Pancrè, V., M. Joseph, C. Mazingue, J. Wietzerbin, A. Capron, and C. Auriault. 1987. Induction of platelet cytotoxic functions by lymphokines: role of interferon-γ. J. Immunol. 138:4490.

19. Pancrè, V., J.Y. Cesbron, C. Auriault, M. Joseph, J. Chandelier, and A. Capron. 1988. IgE-dependent killing of Brugia malayi microfilariae by human platelets and its modulation by T cell products. Int. Arch. Allergy. Appl. Immunol. 85:483.

20. Symer, D.E., T.M. Wright, J. Nishijima, W.A. Paznekas, D.K. Zeiter, and H.S. Shin. 1988. Platelet cytotoxic system capable of specific recognition and lysis of target cells is pre-formed and may include phospholipase A2 activity. FASEB (Fed. Am. Soc. Exp. Biol.) J. 2:A463.

21. Ibele, G.M., N.E. Kay, G.J. Johnson, and H.S. Jacob. 1985. Human platelets exert cytotoxic effects on tumor cells. Blood. 65:1252.

22. Deem, R.L., L.J. Britvan, and S.R. Targan. 1987. Definition of a secondary target cell trigger during natural killer cell cytotoxicity: possible role of phospholipase A2. Cell. Immunol. 110:253.

23. Seaman, W.E. 1983. Human natural killer cell activity is reversibly inhibited by antagonists of lipoygenation. J. Immunol. 131:2953.

24. Villa, M.L., F. Valenti, and M. Mantovani. 1988. Modulation of natural killing by cyclo- and lipo-oxygenase inhibitors. Immunology. 63:93.

25. Cross, P.E., R.P. Dickinson, M.J. Parry, and M.J. Randall. 1986. Selective thromboxane synthetase inhibitors. 2. 3-(1H-imidazol-1-ylmethyl)-2-methyl-1H-indole-1-propanoic acid and analogues. J. Med. Chem. 29:342.

26. Locksley, R.M., J. Fankhauser, and W.R. Henderson. 1985. Alteration of leukotriene release by macrophages ingesting Toxoplasma gondii. Proc. Natl. Acad. Sci. USA. 82:6922.

27. Anderson, S.E., S.C. Bautista, and J.S. Remington. 1976. Specific antibody-dependent killing of Toxoplasma gondii by normal macrophages. Clin. Exp. Immunol. 26:375.

28. Kocsis, J.J., J. Hernandovich, M.J. Silver, J.B. Smith, and C. Ingerman. 1973. Duration of inhibition of platelet prostaglandin formation and aggregation by ingested aspirin or indomethacin. Prostaglandins. 3:141.

29. Purdon, A.D., D. Patelenas, and J.B. Smith. 1987. Evidence for the release of arachidonic acid through the selective action of phospholipase A2 in thrombin-stimulated human platelets. Biochim. Biophys. Acta. 920:205.

30. Kettner, C., and E. Shaw. 1979. D-Phe-Pro-ArgCH₂Cl - a selective affinity label for thrombin. Thromb. Res. 14:969.

31. Needleman, P., S. Moncada, S. Bunting, J.R. Vane, M. Hamborg, and B. Samuelsson. 1976. Identification of an enzyme in platelet microsomes which generates thromboxane A2 from prostaglandin endoperoxides. Nature (London). 261:558.

32. Hammarström, S., and P. Faladeau. 1977. Resolution of prostaglandin endoperoxide synthase and thromboxane synthase of human platelets. Proc. Natl. Acad. Sci. USA. 74:3691.

33. Murray, H.W., and Z.A. Cohn. 1979. Macrophage oxygen-dependent antimicrobial activity. I. Susceptibility of Toxoplasma gondii to oxygen intermediates. J. Exp. Med. 150:938.

34. Henderson, W.R., E.Y. Chi, and S.J. Klebanoff. 1980. Eosinophil peroxidase-induced mast cell secretion. J. Exp. Med. 152:265.

35. Pick, E., and D. Mizel. 1981. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. J. Immunol. Methods. 46:221.

36. Geissler, T., and W.R. Henderson. 1988. Inability of aflatoxin B1 to stimulate arachidonic acid metabolism in human polymorphonuclear and mononuclear leukocytes. Careinogenesis (London). 9:1135.

37. Geissler, T., F.B. Kuzan, E.M. Feustman, and W.R. Henderson, Jr. 1989. Lipid mediator production by post-implantation rat embryos in vitro. Prostaglandins. 38:145.

38. Sabin, A.B., and H.A. Feldman. 1948. Dyes as chemical indicators of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma). Science (Wash. DC). 108:660.

39. Feldman, H.A. 1956. The relationship of Toxoplasma antibody activator to the serum-properdin system. Ann. NY. Acad. Sci. 66:263.

40. Strannegård, Ö. 1967. An electron microscopic study on the immunoinactivation of Toxoplasma gondii. Acta Pathol. Microbiol. Immunol. Scand. 71:463.

41. Anderson, S.E., Jr., and J.S. Remington. 1974. Effect of normal and activated human macrophages on Toxoplasma gondii. J. Exp. Med. 139:1154.

42. Sheffield, H.G., and M.L. Melton. 1968. The fine structure and reproduction of Toxoplasma gondii. J. Parasitol. 54:209.

43. Zucker-Franklin, D. 1981. Megakaryocytes and platelets. In Atlas of Blood Cells. Function and Pathology. Vol. II. D. Zucker-Franklin, M.F. Greaves, C.E. Rossi, and A.M. Marmon, editors. Lea and Febiger, Philadelphia. 559–602.

44. Mills, E.L., J.M. Gerrard, D. Filipovich, J.D. White, and P.G. Quie. 1978. The chemiluminescence response of human platelets. J. Clin. Invest. 61:807.

45. Wörner, P. 1981. Arachidonic acid-induced chemiluminescence of human platelets: contribution of the prostaglandin and lipoxigenase pathways. Thromb. Haemostasis. 46:584.

46. Marcus, A.J., S.T. Silk, L.B. Safer, and H.L. Ullman. 1977. Superoxide production and reducing activity in human platelets. J. Clin. Invest. 59:149.

47. Hamborg, M., J. Svensson, and B. Samuelsson. 1975. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Natl. Acad. Sci. USA. 72:2994.

48. Nicolau, K.C., R.L. Magolda, and D.A. Claremon. 1980. Carboxycyclic thromboxane A2.
generation by the larval cestode, *Taenia taeniaeformis*. Clin. Immunol. Immunopathol. 28:67.

50. Leid, R.W., and L.A. McConnell. 1983. PGE$_2$ generation and release by the larval stage of the cestode, *Taenia taeniaeformis*. Prostaglandins Leukotrienes Med. 11:317.

51. Fusco, A.C., B. Salafsky, and M.B. Kevin. 1985. *Schistosoma mansoni*: eicosanoid production by cercariae. Exp Parasitol. 59:44.

52. Gulbis, E., A.M. Marion, J.E. Dumont, and E. Schell-Frederick. 1979. Prostaglandin formation in bacteria. Prostaglandins. 18:397.

53. Lefer, A.M., E.F. Smith III, H. Araki, J.B. Smith, D. Aharony, D.A. Claremon, R.L. Magolda, and K.C. Nicolaou. 1980. Dissociation of vasoconstrictor and platelet aggregatory activities of thromboxane by carbocyclic thromboxane A$_2$, a stable analog of thromboxane A$_2$. Proc Natl Acad Sci USA. 77:1706.

54. Smith, E.F., III, A.M. Lefer, D. Aharony, J.B. Smith, R.L. Magolda, D. Claremon, and K.C. Nicolaou. 1981. Carbocyclic thromboxane A$_2$: aggravation of myocardial ischemia by a new synthetic thromboxane A$_2$ analog. Prostaglandins. 21:443.

55. Tripp, C.S., K.M. Leahy, and P. Needleman. 1985. Thromboxane synthase is preferentially conserved in activated mouse peritoneal macrophages. J. Clin. Invest. 76:898.

56. Tripp, C.S., P. Needleman, and E.R. Unanue. 1987. Indomethacin in vivo increases the sensitivity to *Listeria* infection in mice. A possible role for macrophage thromboxane A$_2$ synthesis. J. Clin. Invest. 79:399.