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Uptake of apoptotic cells drives the growth of a pathogenic trypanosome in macrophages

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After apoptosis, phagocytes prevent inflammation and tissue damage by the uptake and removal of dead cells[1]. In addition, apoptotic cells evoke an anti-inflammatory response through macrophages[2,3]. We have previously shown that there is intense lymphocyte apoptosis in an experimental model of Chagas' disease[4], a debilitating cardiac illness caused by the protozoan *Trypanosoma cruzi*. Here we show that the interaction of apoptotic, but not necrotic T lymphocytes with macrophages infected with *T. cruzi* fuels parasite growth in a manner dependent on prostaglandins, transforming growth factor-β (TGF-β) and polyamine biosynthesis. We show that the vitronectin receptor is critical, in both apoptotic-cell cytoadherence and the induction of prostaglandin E_2/TGF-β release and ornithine decarboxylase activity in macrophages. A single injection of apoptotic cells in infected mice increases parasitaemia, whereas treatment with cyclooxygenase inhibitors almost completely ablates it *in vivo*. These results suggest that continual lymphocyte apoptosis and phagocytosis of apoptotic cells by macrophages have a role in parasite persistence in the host, and that cyclooxygenase inhibitors have potential therapeutic application in the control of parasite replication and spread in Chagas' disease.

We have already shown that the onset of activation-induced cell death in $CD4^+$ T cells exacerbates parasite replication in co-cultured macrophages infected with *T. cruzi*[5]. To investigate whether the clearance of apoptotic cells predisposes macrophages to *T. cruzi* infection, murine resident peritoneal macrophages were exposed to apoptotic, necrotic or viable splenic T cells first, and then washed and infected. Apoptotic, but not necrotic or living T cells increased *T. cruzi* growth in macrophage cultures (Fig. 1a). Similar results were obtained when apoptotic or necrotic cells were added after *T. cruzi* infection (data not shown). Nevertheless, treatment of lymphocytes with the caspase-inhibitor zVAD-fmk peptide before apoptosis induction, rescued T cells from death (data not shown) and prevented the increase in parasite replication (Fig. 1b) in a dose-dependent manner. In another model, peritoneal macrophages from mice infected with *T. cruzi* were incubated with apoptotic or necrotic cells. Apoptotic, but not necrotic cells also exacerbated endogenous *T. cruzi* growth in these *in vivo* infected macrophages (Fig. 1c). In agreement with *in vitro* results, a single *in vivo* injection of apoptotic, but not necrotic cells in *T. cruzi*-infected mice resulted in a sudden rise in parasitaemia (Fig. 1d).

Previous studies pointed to a role for an integrin in the recognition and ingestion of apoptotic cells by macrophages[6,7]. We observed that RGDS, but not RGES peptide blocked apoptotic cell binding and reduced *T. cruzi* growth within macrophages (data not shown).

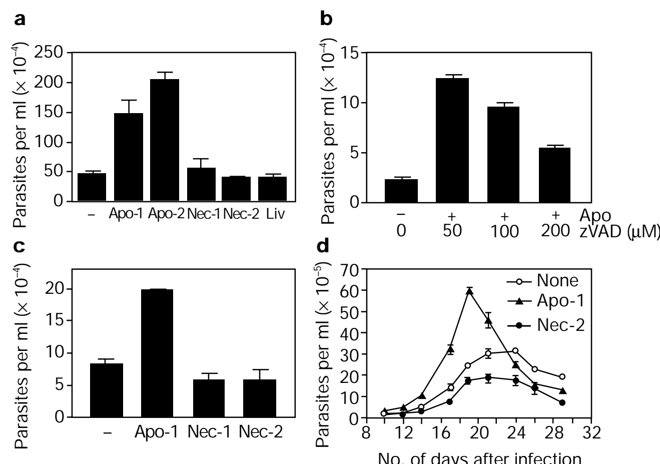

Figure 1 Apoptotic cells exacerbate parasite growth in *T. cruzi* infection. **a**, Peritoneal resident macrophages were either untreated (–) or exposed to Apo-1, Apo-2, Nec-1, Nec-2 or to living cells (Liv). After 5 days, cells were removed and macrophages were infected with *T. cruzi*; parasites were counted 10 days later. **b**, T cells (Apo) were treated with the indicated doses of zVAD-fmk (zVAD) before apoptosis induction (by heating) and added to macrophages infected *in vitro*. Parasites were counted 7 days later. Maximal cell death (100%) and increased parasite replication still occurred with zVAD-fmk at 50 μM. **c**, Macrophages from infected mice were exposed to Apo-1, Nec-1 or Nec-2 throughout culture, and trypomastigotes counted 10 days later. **d**, Mice were injected 7 days after infection with Apo-1 or Nec-2, and parasitaemia was monitored.

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The vitronectin receptor (VnR) integrin ($\alpha_v\beta_3$, CD51/CD61) is involved in phagocytosis of apoptotic cells by macrophages[6,7]. Both VnR and CD36 take part in the macrophage receptor that bridges thrombospondin exposed on apoptotic cells[7]. Flow cytometry analysis revealed that both α_v- and β_3-integrin chains were absent from the surface of control resident macrophages, but their expression was upregulated in the course of *T. cruzi* infection (Fig. 2a, b). In addition, $\alpha_v\beta_3$ expression was induced in control macrophages after a 24-h culture (not shown). We therefore investigated the role of VnR in the binding of apoptotic cells by *in vivo* infected macrophages. After a 3-h incubation, nearly all macrophages were rosetted by apoptotic cells (mean 93.5 ± 2.5% positive macrophages). Both intact monoclonal antibodies against α_v- or β_3-VnR chains and anti-α_v fragment of antigen binding (Fab) inhibited apoptotic cell binding by 40–50% (Fig. 2c).

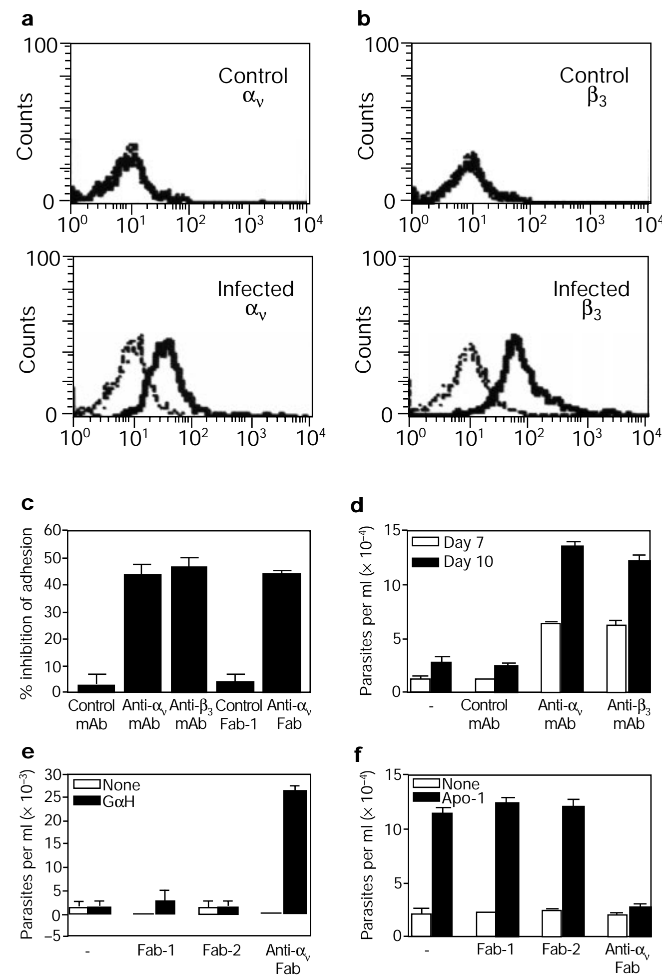

Figure 2 Effects of apoptotic cells on macrophages infected with *T. cruzi* are mediated by the vitronectin receptor. α_v (**a**) and β_3 (**b**) VnR chains are expressed by macrophages from infected (lower panels), but not uninfected (upper panels), mice. Mac-1$^+$ cells were gated and analysed for α_v (**a**) or β_3 (**b**) expression. Bold lines, anti-α_v (**a**) or anti-β_3 (**b**) staining; dotted lines; cells stained after treatment with unlabelled anti-α_v (**a**) or anti-β_3 (**b**) monoclonal antibodies; dashed lines, unstained cells. **c**, Anti-VnR monoclonal antibodies and Fab inhibit apoptotic cell binding to macrophages. Macrophages infected *in vivo* were treated with anti-α_v Fab or control anti-CD69 Fab-1, anti-α_v, anti-β_3 or control hamster IgG monoclonal antibodies (mAb) (20 min), and then exposed to Apo-1. Adhesion was determined after 3 h. **d**, **e**, Anti-α_v or anti-β_3, but not control monoclonal antibody drives *T. cruzi* replication within macrophages. Macrophages infected *in vivo* were treated with monoclonal antibodies (**d**) or were treated first with anti-α_v Fab, Fab-1 or control hamster IgG Fab-2 (50 μg ml^{-1}) for 20 min, washed and treated with goat anti-hamster IgG antibody (GαH) at 10 μg ml^{-1} (**e**). Parasites were counted as indicated (**d**) or after 10 days (**e**). **f**, Anti-α_v Fab inhibits the apoptotic cell effects on *T. cruzi* replication. Macrophages infected *in vivo* were treated first with Fab fragments (20 min) and then with Apo-1. Parasites were counted 10 days later.

We then investigated VnR involvement in parasite replication. Engagement of VnR by anti-α_v or anti-β_3 monoclonal antibodies, either soluble (Fig. 2d) or immobilized on plates (Fig. 3c), markedly increased *T. cruzi* growth in macrophages in the absence of apoptotic cells. Soluble anti-α_v Fab fragments failed to enhance parasite replication, but promoted *T. cruzi* growth upon crosslinkage by a secondary goat anti-hamster IgG (Fig. 2e). Most notably, soluble anti-α_v Fab completely blocked the apoptotic cell effect on parasite replication in macrophages obtained from infected mice (Fig. 2f). A control hamster anti-CD69 Fab had no effect (Fab-1 in Fig. 2f), even though it completely inhibited CD69 staining in the macrophages used in these experiments (data not shown). Overall, these results show that blockade of VnR was sufficient to ablate parasite replication, whereas VnR crosslinkage mimicked the effects of apoptotic cells on *T. cruzi* growth in macrophages.

Interaction with apoptotic cells leads macrophages to secrete TGF-β, which in turn suppresses their pro-inflammatory cytokine response[3]. In addition, TGF-β renders both phagocytic and non-phagocytic cells permissive to *T. cruzi* infection[8,9] and antagonizes interferon (IFN)-γ-induced nitric oxide (NO) production and trypanocidal activity of macrophages[10]. We investigated the role of TGF-β in *T. cruzi* infection in macrophages treated with apoptotic cells. Both uninfected and infected macrophages produced similar levels of TGF-β in response to apoptotic, but not necrotic, cells (Fig. 3a). We also detected marked secretion of TGF-β triggered by anti-α_v monoclonal antibody (Fig. 3b) in the absence of *T. cruzi* infection and apoptotic cells. Anti-α_v Fab promoted TGF-β production only in the presence of a secondary goat anti-hamster IgG (Fig. 3b), but completely ablated TGF-β secretion in response to apoptotic cells (data not shown). We treated infected macrophages with anti-TGF-β1 neutralizing antibody. Neutralization of TGF-β inhibited parasite replication induced by either apoptotic cells or immobilized anti-β_3 monoclonal antibody (Fig. 3c), whereas a control IgY antibody had no effect. Although the involvement of other co-receptors or co-factors[2] cannot be ruled out, the present data indicate that engagement of VnR by apoptotic cells may result in a TGF-β-dependent increase in parasite replication in macrophages. We also investigated whether apoptotic cells and TGF-β interfere with NO-dependent trypanocidal activity of macrophages. Apoptotic cells blocked NO production by IFN-γ/LPS-activated macrophages (Fig. 3d, left) and led to vigorous *T. cruzi* replication (Fig. 3d, right). Inhibition of NO production by apoptotic cells could be reversed by anti-TGF-β antibodies or anti-α_v Fab (Fig. 3e). Therefore, the uptake of apoptotic cells renders macrophages refractory to inflammatory cytokines, allowing parasite survival and growth even in the face of immune response.

Transforming growth factor-β shifts arginine metabolism in macrophages, decreasing NO and inducing ornithine production (by arginase) and subsequent polyamine biosynthesis[11]. Ornithine decarboxylase (ODC) catalyses putrescine synthesis from ornithine, and is considered as the limiting step in the polyamine biosynthetic pathway, leading to spermidine and spermine production[12]. Although ODC activity has not been identified in *T. cruzi*, intra- and extracellular forms of *T. cruzi* synthesize polyamines either through the alternative agmatine pathway[13] or by using exogenously added putrescine[14]. To investigate the possibility that macrophage putrescine synthesis is involved in the apoptotic cell effects that we observed, putrescine content and ODC activity were measured in macrophages treated with apoptotic cells. Macrophages treated with apoptotic cells markedly accumulated putrescine (Fig. 3f, left), and exogenous addition of putrescine alone increased parasite replication in macrophages (Fig. 3f, right). ODC activity was markedly upregulated in macrophages exposed to apoptotic cells or to immobilized anti-α_v monoclonal antibody, with delayed kinetics

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(Fig. 3g). Both anti-α_v Fab and anti-TGF-β antibodies suppressed apoptotic cell effects on ODC activity (Fig. 3h). A competitive ODC inhibitor, α-methylornithine[15], also blocked ODC activity induced by apoptotic cells (Fig. 3g). Addition of this inhibitor to macrophages treated with apoptotic cells or anti-α_v resulted in a dose-dependent decrease in parasite replication (Fig. 3i), but it had no inhibitory effect on basal *T. cruzi* growth in macrophages left without stimuli (Fig. 3i), and did not affect the uptake of apoptotic cells or TGF-β production (data not shown). These results indicate that TGF-β, produced upon engagement of VnR by apoptotic cells, may induce ODC activity in macrophages and promote polyamine-dependent parasite growth.

Searching therapeutic targets to prevent apoptotic cell effects, a role for prostaglandins on *T. cruzi* growth was investigated. Prostaglandin E_2 and PAF have been reported to induce TGF-β production by human macrophages exposed to apoptotic neutrophils[3]; prostaglandin E_2 increases arginase[16] and ODC[17] activities, and promoted *T. cruzi* growth (data not shown) in macrophages. We therefore evaluated PGE_2 production by macrophages exposed to either apoptotic cells or immobilized anti-α_v monoclonal antibodies. Apoptotic cells induced PGE_2 production by macrophages, and this effect was prevented by anti-α_v Fab but not by control anti-CD69 Fab-1 (Fig. 4a). Vitronectin receptor ligation by immobilized anti-α_v monoclonal antibodies also increased PGE_2 levels, whereas anti-CD69 or anti-Mac-1 (α_M/β_2 integrin) control monoclonal antibodies had no effect (Fig. 4a). We tested three non-steroidal anti-inflammatory drugs (NSAIDs): aspirin, an inhibitor of both constitutive and inducible cyclooxygenases (COX); indomethacin, a preferential antagonist of constitutive COX[18]; and NS-398, an inducible COX inhibitor[19]. Indomethacin almost completely blocked anti-VnR induced PGE_2 (Fig. 4a) and TGF-β (data not shown) production. Indomethacin, aspirin and NS-398 significantly suppressed apoptotic cell effects on PGE_2 (Fig. 4b) and TGF-β (data not shown) secretion, on ODC activity *in vitro* (Fig. 4c) and *in vivo* (Fig. 4d), and on *T. cruzi* growth, without affecting basal parasite replication (Fig. 4e). Furthermore, COX

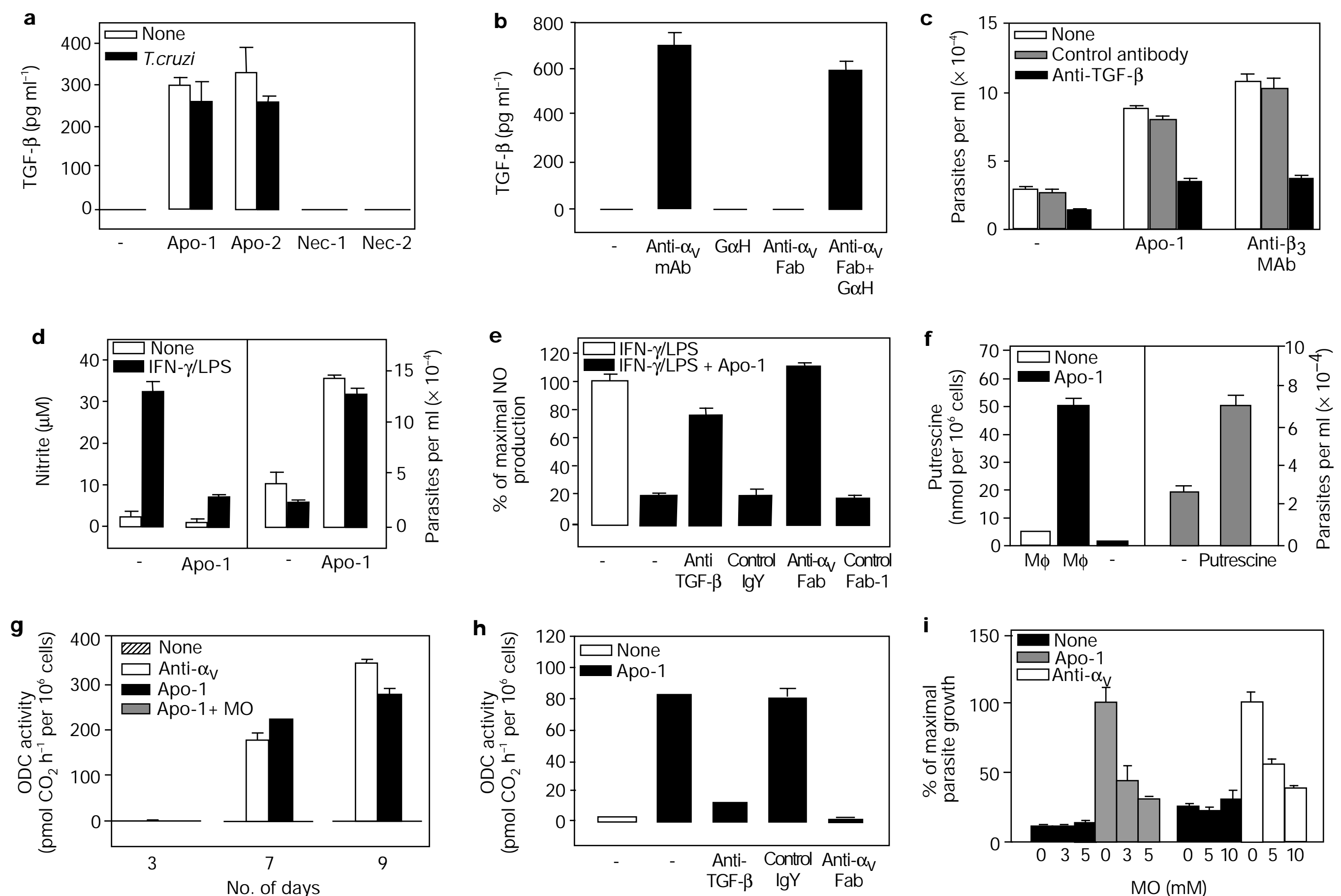

Figure 3 Effects of apoptotic cells on macrophages infected with *T. cruzi* depend on TGF-β. **a**, Apoptotic (Apo-1, -2), but not necrotic (Nec-1, -2), cells induce TGF-β production by uninfected (open bars) or *in vitro* infected (closed bars) macrophages TGF-β1 was evaluated in supernatants from 48-h cultures. **b**, VnR engagement induces TGF-β production by macrophages. Uninfected macrophages were exposed to anti-α_v monoclonal antibody (mAb) or Fab, followed or not by goat anti-hamster IgG (GαH), and TGF-β1 was evaluated in supernatants from 24-h cultures. **c**, Neutralization of TGF-β ablates the effect of apoptotic cells or anti-VnR mAb on macrophages infected with *T. cruzi*. *In vivo* infected macrophages were exposed to Apo-1 or to immobilized anti-β_3 mAb in the presence of either anti-hTGF-β1 (6 μg ml^{-1}) or control IgY antibodies. Trypomastigotes were counted after 10 days. **d**, **e**, Apoptotic cells suppress NO production by LPS/IFN-γ-activated macrophages. *In vivo* infected (**d**) or uninfected (**e**) macrophages were exposed to LPS plus IFN-γ and treated with Apo-1, anti-TGF-β or control IgY antibody, anti-α_v Fab or anti-CD69 Fab-1. Supernatants were assayed for nitrite content after 48 h (**d**, left, **e**) and for parasites after 10 days (**d**, right). In **e**, results were expressed as a percentage of the maximal nitrite content in macrophages treated with LPS plus IFN-γ. **f**, Apoptotic cells (Apo-1) induce putrescine accumulation in uninfected macrophages (Mϕ) and exogenous putrescine (1 mM) increases parasite growth in macrophages infected *in vitro*. Putrescine (left) and parasite accumulation (right) were measured after 7 days. **g**, Kinetics of ODC induction. Macrophages were exposed to immobilized anti-α_v or to Apo-1. ODC inhibitor MO was added at 10 mM. ODC activity was measured at the indicated days. **h**, Apoptotic cell effects on ODC activity depend on TGF-β. Macrophages were exposed to Apo-1 in the presence of anti-α_v Fab, anti-TGF-β or control IgY antibodies. ODC activity was evaluated after 7 days. **i**, ODC activity is required for *T. cruzi* growth. *In vitro* infected macrophages were exposed to Apo-1 or to anti-α_v mAb, and MO was added at the indicated dosages. Trypomastigotes were counted after 8 (anti-α_v) or 10 (Apo-1) days in culture and are expressed as a percentage of the maximal parasite growth in the absence of MO.

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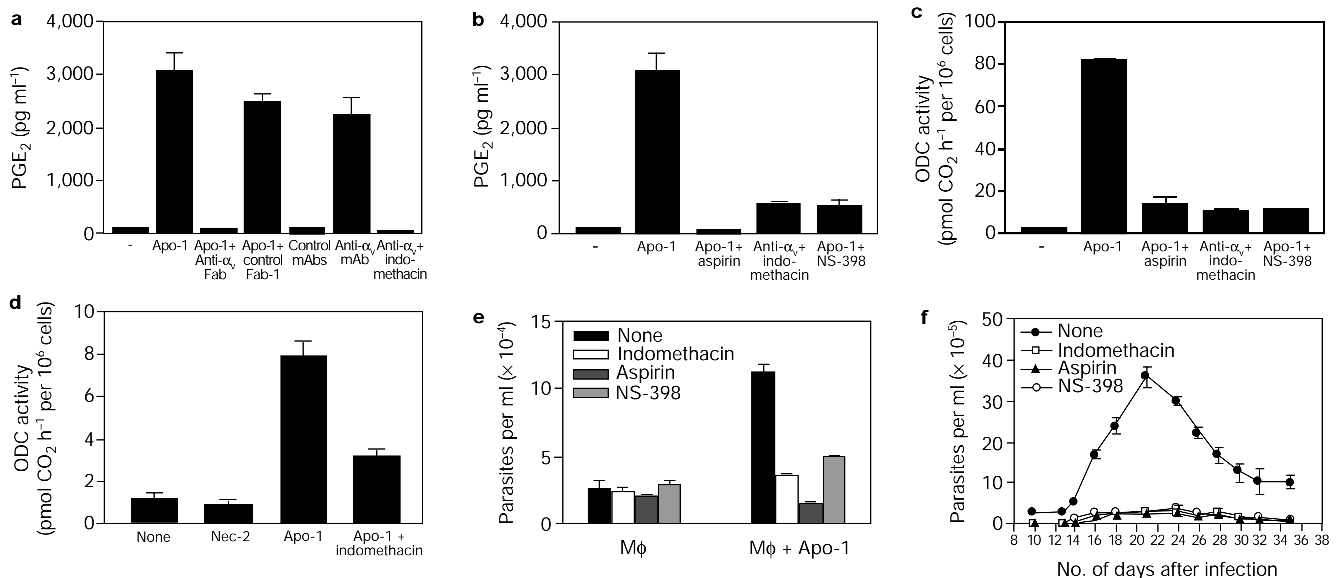

Figure 4 *In vitro* and *in vivo* effects of COX inhibitors on *T. cruzi* infection. **a**, Apoptotic cells or VnR ligation induce PGE_2 release by macrophages. Macrophages were treated with Apo-1, anti-α_v Fab or anti-CD69 Fab-1, immobilized anti-α_v, anti-CD69 or anti-Mac-1 mAbs, or anti-α_v plus indomethacin. PGE_2 was measured as described. For simplicity, results with anti-CD69 and anti-Mac-1 were combined in the figure (control mAbs). **b**, **c**, COX inhibitors block PGE_2 release and ODC activity *in vitro*. Macrophages were exposed to Apo-1 and treated with aspirin, indomethacin or NS-398. PGE_2 levels were measured after 24 h (**b**), and ODC activity was assayed after 7 days (**c**). **d**, Apoptotic cells induce ODC activity *in vivo*. Mice ($n = 3$) were injected i.p. with Nec-2, Apo-1 or Apo-1 plus indomethacin. ODC activity was measured 10 days later in adherent PEC. **e**, COX inhibitors antagonize apoptotic cell effects on macrophages infected with *T. cruzi*. *In vitro* infected macrophages (Mϕ) were exposed to Apo-1 and treated with COX inhibitors as in **b**. Trypomastigotes were counted after 7 days. **f**, COX inhibitors suppress parasitaemia in *T. cruzi* infected mice. Mice ($n = 4$) were infected with *T. cruzi* and treated 7, 8 and 9 days after infection with aspirin, indomethacin or NS-398. Parasitaemia was monitored throughout acute infection.

inhibitors almost completely abolished parasitaemia in infected mice, after a brief 3-day treatment, one week after infection (Fig. 4f). These data indicate that the factors driving *T. cruzi* replication *in vivo* may be the same mediators as are involved in the parasite growth-promoting activity of apoptotic cells *in vitro*. However, these results should be taken with caution, as NSAIDs can have differential effects on macrophages depending on dosage[20]. More experiments are necessary to understand the discrepant effects of high-dose therapy with COX inhibitors seen in other reported studies on *T. cruzi* infection[21].

Our results show that VnR engagement by apoptotic cells triggers PGE_2 and TGF-β release by macrophages. The suppressive effect of PGE_2/TGF-β on pro-inflammatory cytokine expression[3] and NO production would create the appropriate environment for optimal *T. cruzi* growth within macrophages. Furthermore, the uptake of apoptotic cells reorientates macrophage metabolism towards putrescine production, helping parasite replication. We have shown that widely used drugs, such as aspirin and indomethacin, interfere with this pathway and are able to control parasitaemia in susceptible mice, making them potentially useful for therapy in the acute phase of Chagas' disease. □

Methods

Animals and infection

We infected BALB/c male mice (6 weeks of age) intraperitoneally (i.p.) with 10^4 metacyclic trypomastigote forms of *T. cruzi* clone Dm 28c (ref. 22).

Macrophages

Peritoneal exudate cells (PEC) removed from the peritoneal cavity of uninfected or acutely infected mice (3–4 weeks after infection) were cultured in complete medium (DMEM, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 μg ml^{-1} gentamicin, MEM non-essential amino acids, 10 mM Hepes and 50 μM 2-ME) with 1% Nutridoma-SP (Boehringer Mannheim) or 5% FCS (experiments in Fig. 1a, c) at 3×10^5 cells ml^{-1} on 24-well plates (Corning). Macrophages from normal mice were infected with 1.5×10^6 *T. cruzi* metacyclic forms per well[5]. After 24 h, non internalized parasites were removed and macrophages cultured in complete medium (1 ml) at 37 °C, with 7% CO_2 for up to 10 days. Extracellular motile trypomastigotes were counted in culture supernatants[5].

Lymphocytes

Nylon-wool-filtered normal splenocytes were suspended in complete medium and heated (56 °C for 30 min) (Apo-1) or irradiated with 30 Gy (Apo-2) to obtain apoptotic cells as described[23]. Cells were also fixed with 1% paraformaldehyde for 20 min at room temperature (followed by extensive washing) (Nec-1) or frozen-thawed (Nec-2) to obtain necrotic cells[23]. Dead or viable cells (10^6 per well) were then added to macrophages.

Antibodies and reagents

Anti-β_3 integrin (CD61), PE-labelled anti-β_3 and anti-α_v (CD51), control hamster IgG, unlabelled and FITC- and PE-labelled anti-Mac-1, FITC-labelled anti-CD69, and anti-CD16/CD32 monoclonal antibodies were purchased from Pharmingen (San Diego). Purified chicken anti-hTGF-β1 IgY was from R&D Systems (Minneapolis) and control (chicken anti-canine IgG) IgY antibody (a gift from V. Laurentino) was prepared as described[24]. Anti-α_v mAb H9.2B8 (ref. 25), and anti-CD69 mAb H1.2F3 (ref. 26) were donated by E. Shevach. Fab fragments of control hamster IgG, anti-CD69 and anti-α_v monoclonal antibodies were produced with a commercial Fab preparation kit (Pierce). zVAD-fmk peptide was a gift from M. Lenardo. Other reagents were goat anti-hamster IgG antibody (Cappel-Organon Teknika Corporation), mrIFN-γ (Pharmingen), LPS (*Escherichia coli* O111:B4; Difco), α-methil-ornithine, MO (Marion Merrel Dow), Putrescine (Sigma), aspirin and indomethacin (Sigma), and NS-398 (Biomol).

Adhesion assay

Macrophages were cultured with Apo-1 and anti-α_v, anti-β_3 or control hamster IgG mAbs (10 μg ml^{-1}), anti-α_v Fab or control anti-CD69 Fab-1 (50 μg ml^{-1}) for 3 h, washed, fixed with 1% paraformaldehyde, and counted under phase-contrast microscopy. Adhesion was calculated as the percentage of rosetted macrophages out of a total of 100 macrophages counted per well.

TGF-β production

Macrophages were treated with apoptotic or necrotic cells, or with anti-α_v monoclonal antibody or Fab plus goat anti-hamster IgG (10 μg ml^{-1}). The content of TGF-β1 in supernatants was evaluated by sandwich ELISA as described[27].

NO production

Macrophages were cultured with IFN-γ (40 U ml^{-1}) plus LPS (10 ng ml^{-1}), and exposed or not to Apo-1, anti-TGF-β or control IgY antibody (6 μg ml^{-1}), anti-α_v Fab or control anti-CD69 Fab-1. Supernatants were collected 48 h later and mixed with an equal volume of Griess reagent to determine nitrite content, as described[28].

PGE_2 release

Macrophages were exposed to immobilized anti-α_v or control monoclonal antibodies (10 μg ml^{-1}), Apo-1, Fab fragments, aspirin (10 μg ml^{-1}), indomethacin (1 μg ml^{-1}) or

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NS-398 (1 μM). Culture supernatants (24 h) were collected and assayed for PGE_2 using a competitive ELISA kit (Cayman Chemicals).

ODC activity and putrescine content

Macrophages were cultured (10^6 per ml) with Apo-1 (3×10^6 per well), anti-α_v monoclonal antibody, MO or other reagents. Ornithine decarboxydase activity and putrescine content were evaluated as described[29].

Flow cytometry

Peritoneal exudate cells (PEC) were treated with F_c block (anti-CD16/CD32) and stained with FITC-labelled anti-Mac-1 plus PE-labelled anti-α_v or anti-β_3 monoclonal antibodies. Unlabelled anti-α_v or anti-β_3 monoclonal antibodies were used to block staining specifically. Uninfected macrophages were also cultured for 24 h, detached and stained. Antibodies were used at 1 μg per 10^6 cells. 10^4 cells were acquired, and Mac-1-positive cells were gated and analysed for either α_v or β_3 expression on a B-D Xcalibur flow cytometer.

In vivo experiments

Infected mice ($n = 4$) were injected i.p. with 10^7 Apo-1 or Nec-2, 7 days after infection, or left without treatment. Parasitaemia was determined on blood samples from the tail. In other experiments, infected mice ($n = 4$) were injected i.p. at 7, 8 and 9 days after infection, with aspirin (50 mg kg^{-1}), indomethacin (1 mg kg^{-1}) or NS-398 (5 mg kg^{-1}), or left untreated, and parasitaemia was followed. For *ex-vivo* determination of ODC activity, mice ($n = 3$) were injected i.p. with 10^7 Apo-1 or Nec-2, or left without treatment. Mice injected with Apo-1 were untreated or treated with indomethacin (1 mg kg^{-1}) in the same day, and 4 days later. ODC activity was measured in adherent PEC (10^6 per well) 10 days later.

Presentation of results and statistics

Each experiment presented is representative of at least three independent experiments. Data are expressed as mean ± s.e. of duplicate determinations. For *in vivo* experiments, results are expressed as mean ± s.e. of individual animals. Significance was evaluated by Student's unpaired *t*-test, and all positive results mentioned were significant ($P < 0.05$ or <0.01) compared with controls.

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The major protein import receptor of plastids is essential for chloroplast biogenesis

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Light triggers the developmental programme in plants that leads to the production of photosynthetically active chloroplasts from non-photosynthetic proplastids[1]. During this chloroplast biogenesis, the photosynthetic apparatus is rapidly assembled, mostly from nuclear-encoded imported proteins[2–4], which are synthesized in the cytosol as precursors with cleavable amino-terminal targeting sequences called transit sequences. Protein translocon complexes at the outer (Toc complex)[5–7] and inner (Tic complex)[6,8,9] envelope membranes recognize these transit sequences, leading to the precursors being imported. The Toc complex in the pea consists of three major components, Toc75, Toc34 and Toc159 (formerly termed Toc86)[6,7,10,11]. Toc159, which is an integral membrane GTPase[12], functions as a transit-sequence receptor[5–7,13]. Here we show that *Arabidopsis thaliana* Toc159 (atToc159) is essential for the biogenesis of chloroplasts. In an *Arabidopsis* mutant (*ppi2*) that lacks atToc159, photosynthetic proteins that are normally abundant are transcriptionally repressed, and are found in much smaller amounts in the plastids, although *ppi2* does not affect either the expression or the import

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