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The Murine Antiapoptotic Protein A1 Is Induced in Inflammatory Macrophages and Constitutively Expressed in Neutrophils

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Myeloid leukocytes are thought to regulate their susceptibility to apoptosis upon migration to a site of inflammation. However, factors that determine survival have not been well characterized in these cells. We have examined the expression of murine A1, an antiapoptotic Bcl-2 relative found in activated myeloid cells, during the course of an acute inflammatory response. Intraperitoneal infection of mice with the virulent RH strain of Toxoplasma gondii led to a 5- to 10-fold increase in A1 mRNA levels in peritoneal cells after several days. Bcl-2 expression was unchanged. The increase in A1 expression depended on the dose of the organism and coincided with a sharp increase in peritoneal cellularity. A1 protein levels were also increased as determined by Western blot analysis and immunohistochemical studies. All neutrophils and approximately half of the macrophages in the inflammatory exudate contained high levels of A1 in cytoplasm. A1 expression did not correlate with intracellular parasitization. Peripheral blood neutrophils from normal mice strongly expressed A1 protein, whereas normal monocytes showed only weak staining. Bax mRNA was induced in parallel with A1 in macrophages. Exudate macrophages and granulocytes that were apoptotic by TUNEL staining occasionally appeared to display A1 throughout the cell nucleus. These studies identify A1 as a potential regulator of apoptosis during acute inflammation. The Journal of Immunology, 1999, 163: 412-419.

The accumulation of leukocytes at a site of acute inflammation is governed by a minimum of two processes: emigration of cells from the circulation and removal of infiltrated cells by either drainage or cell death. Although it is well established that leukocyte emigration is a highly regulated process, it is less clear whether this is also the case for cellular removal. However, several lines of evidence now indicate that apoptotic cell death is an important mechanism for the clearance of inflammatory myeloid leukocytes and that regulation of this process plays a critical role in shaping the inflammatory response. First, neutrophils (1–3), eosinophils (4), and macrophages (5–9) have all been observed to undergo apoptosis during acute inflammation, and at least for neutrophils, evidence suggests that this may be the major mode of clearance of extravasated cells (3). Second, leukocyte apoptosis appears to be regulated during inflammation. Neutrophils harvested from inflammatory sites show either decreased (10–12) or increased (2) rates of apoptosis upon culture relative to control circulating cells. Similarly, peritoneal macrophages elicited by infection with Toxoplasma gondii show highly variable rates of apoptosis in culture depending on the strain of parasite (13). Third, a wide variety of cytokines, hormones, inflammatory mediators, pathogens, and cellular processes have been shown to regulate the life span of both granulocytes and macrophages in vitro (14–30). Finally, the accumulation of inflammatory granulocytes can be either enhanced (2) or reversed (31) by manipulations that, respectively, retard or promote apoptosis in the relevant cells in vivo.

The Bcl-2 family comprises ~20 proteins (bcl2s) that either promote or block apoptosis (32). However, there is little information about the expression of these proteins in inflammatory myeloid leukocytes. Neutrophils and macrophages express Bax, a death-promoting bcl2 (33, 34). Bax can be antagonized by protective bcl2s; however, no protective bcl2s have previously been identified in neutrophils. In macrophages, protective bcl2s have generally not been detected in inflammatory or resident cells in situ. However, Bcl-xL, a protective bcl2, can be induced in cultured macrophages (35), and there is one report of Bcl-2 expression in macrophages in endometriosis (36).

We have previously described a protective murine bcl2, A1, that is rapidly induced in macrophages by either LPS or GM-CSF. In addition, A1 is expressed in promyeloid cells that have been induced to differentiate into neutrophils by G-CSF (37, 38). A1 can interact with Bax (39, 40), indicating a potential mechanism for protection. We investigated A1 expression in a model of an acute inflammatory response to an intracellular protozoan pathogen, T. gondii. The results indicate that A1 is highly expressed in infiltrating macrophages and also in both normal circulating and inflammatory neutrophils.

Materials and Methods

Generation of inflammation in mice

Female BALB/c mice, 6–8 wk old, were obtained from The Jackson Laboratory (Bar Harbor, ME). In some experiments, female outbred CD1 mice 5–7 wk old from Charles River (Wilmington, MA) were used. For some experiments, BALB/c mice were maintained under specific pathogen-free conditions in the barrier facility maintained by the Institute for Animal Studies at Albert Einstein College of Medicine. The RH strain (Sabin) of T. gondii is rapidly induced in macrophages by either LPS or GM-CSF. In addition, A1 is expressed in promyeloid cells that have been induced to differentiate into neutrophils by G-CSF (37, 38). A1 can interact with Bax (39, 40), indicating a potential mechanism for protection. We investigated A1 expression in a model of an acute inflammatory response to an intracellular protozoan pathogen, T. gondii. The results indicate that A1 is highly expressed in infiltrating macrophages and also in both normal circulating and inflammatory neutrophils.

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3 Abbreviations used in this paper: bcl2, Bcl-2 family member; PBS-CMF, calcium- and magnesium-free PBS; PBS-A, PBS-CMF containing 0.2% BSA; p.i., postinfection.


**Harvest and extraction of cells**

For peritoneal lavage, mice were sacrificed by cervical dislocation, and the peritoneal cavity was washed with 4 ml of ice-cold PBS-CMF containing 0.2 % BSA (low endotoxin grade; Sigma, St. Louis, MO) (PBS-A). Cellularity and extracellular tachyzoites were assessed with a hemacytometer. Blood was collected by cardiac puncture following CO2 anesthesia. Blood from each individual mouse or pools of seven mice was collected onto an equal volume of ice-cold isotonic saline containing 10 U/ml heparin and 10 mM EDTA. All further steps were at 0–4°C except as indicated. Blood was aspirated with Ficol/sodium diatrizoate (density, 1.090) and centrifuged at 600 × g for 13 min, and total leukocytes were recovered from the interface. The neutrophil count in this leukocyte fraction was similar to reported values (data not shown). The interface was diluted with 5–10 vol PBS-A, centrifuged at 150 × g for 10 min, and resuspended in PBS-A. For some experiments, peritoneal cells were washed with PBS-A, brought to room temperature, and separated by centrifugation at room temperature over Histopaque, 1.077 g/ml (Sigma), as described for blood. Samples of blood leukocytes and peritoneal cells were cytocentrifuged (700 rpm, 5 min). Slides were either air dried, fixed in methanol, and stained with Diff-Quik (Dade International, Miami, FL) for differential counting or fixed in cold paraformaldehyde (4% in PBS-CMF) for 20 min, washed several times with PBS-CMF, and stored in PBS-CMF at 5°C for up to 10 days before immunostaining. Intracellular parasitization was assessed in Diff-Quik-stained preparations by examination of 500 macrophages under oil immersion. For extraction of total cellular RNA, cell suspensions were centrifuged (150 × g, 10 min), resuspended in residual volume, lysed with Trizol (Boehringer Mannheim, Indianapolis, IN), and extracted according to the manufacturer’s protocol. For extraction of total cellular protein, cell suspensions were additionally washed in PBS to remove BSA, extracted with a modification of Laemmli gel sample buffer (38), boiled for 5 min, and stored at −20°C.

**Northern blot analysis**

Total cellular RNA (2–4 μg) was precipitated with sodium acetate and ethanol, separated on 1.2% agarose-formaldehyde gels, and transferred to nylon membranes (either Hybond-N (Amersham, Arlington Heights, IL) or Biodyne A (Life Technologies, Gaithersburg, MD)) as previously described (38). Prehybridization, hybridization (in 50% formamide at 42°C), washing of filters were as described (42). The A1 probe was an EcoRI fragment from the pBlueA1 cDNA plasmid previously described (38). The probes for murine Bcl-2 and Bax were kind gifts of Dr. J. C. Reed (The Burnham Institute, La Jolla, CA). The probe for GM-CSF can be prehybridized at hybridization (in 50% formamide at 42°C). The levels of A1 and Bcl-2 gene expression in the inflammatory infiltrate were assessed by Northern blot analysis. As shown in Fig. 2, a dramatic increase in A1 expression occurred at about day 3 p.i. and was maintained for the duration of the experiment. In comparison, the level of Bcl-2 mRNA was unchanged. The leukocyte influx was accompanied by a steady increase in both extracellular and intracellular parasites (Fig. 1, C and D). Infection resulted in 100% mortality at −1 wk p.i. (data not shown).

**Preparation of an anti-peptide antisemur**

A peptide corresponding to residues 1–16 of the murine A1 sequence, with an additional C-terminal cysteine, was synthesized by the Laboratory of Macromolecular Analysis at the Albert Einstein College of Medicine. The peptide was conjugated to maleimide-activated keyhole limpet hemocyanin (Pierce, Rockford, IL) according to the manufacturer’s protocol. A rabbit antisemur against this conjugate was prepared by Anascpe (San Jose, CA). Rabbits were injected with conjugate (>50 μg peptide) + CFA and boosted at 3, 6, and 10 wk with conjugate + IFA. Bleeds were screened initially by ELISA for the production of a peptide-BSA conjugate and then by Western blot analysis of A1-transfected COS cells. For affinity purification, the serum was diluted in Tris-buffered saline (pH 7.4) and applied to a peptide-agarose column prepared by the covalent linkage of residues 1–16 using the Sulfolink method (Pierce) according to the manufacturer’s protocol. The column was washed with Tris-buffered saline and sequentially eluted first with 3 M potassium thiocyanate and then with 0.1 M glycine (pH 2.5). Eluates were neutralized with 0.1 vol 1 M Tris-HCl (pH 7.5) and dialyzed against PBS-CMF and their specificity was verified by analysis of Western blots containing either mock or A1-transfected COS cells (R. D. Somogyi et al., manuscript in preparation).

**Immunocytochemistry**

Fixed cytosin preparations were permeabilized in 0.2% Triton X-100 in PBS-CMF for 25 min. Slides were rinsed in PBS-CMF, and endogenous peroxidase was blocked by incubation in 1 mM sodium azide, 10 mM glucose, and 1% (w/v) glucose oxidase (Sigma) in PBS-CMF for 1 h at 37°C (46). The slides were rinsed with PBS-CMF and blocked for 30 min in PBS-CMF containing 0.05% Triton X-100, 2% BSA, and 1% goat serum. The slides were then probed with the 855AP Ab (1:100 in the block buffer) for 2 h. The cells were washed four times with PBS-CMF + 0.05% Tween-20, and the signal was detected by the Ultrasensitive ABC method (Pierce) according to the supplier’s protocol using diaminobenzidine. For dual detection of apoptosis and A1, fixed cytosin preparations were initially probed for nicked DNA by TUNEL using a kit from Boehringer Mannheim according to the manufacturer’s protocol. The slides were then washed with PBS-CMF containing 0.05% Triton X-100 and blocked and probed with 855AP Ab, as above. The slides were then washed with PBS-CMF + 0.05% Triton X-100, the blocking step was repeated, and Texas Red-X goat anti-rabbit IgG (H+L) (Molecular Probes, Eugene, OR) was applied at 10 μg/ml for 2 h. Slides were mounted using the ProLong antifade kit (Molecular Probes) and examined by epifluorescence using 13, N2.1, or G/R filters (Leica, Deerfield, IL). Images were scanned into Adobe Photoshop 5.0.
In comparison, A1 expression in thioglycolate-elicited peritoneal cells (predominantly macrophages) was also significantly elevated (Fig. 4), although the increase (2.3-fold) was less than that seen with *T. gondii* infection (7.1-fold in this experiment). Very similar expression was observed at day 3 and day 6 of thioglycolate stimulation (data not shown). Vehicle-injected animals showed no increase in A1.

**A1 is expressed in inflammatory macrophages and neutrophils**

To examine A1 protein expression in individual cells, an affinity-purified Ab (855AP) was prepared against a peptide corresponding to the A1 N terminus. The ability of this Ab to recognize A1 specifically was verified by Western blot analysis (Fig. 5). A species comigrating with A1 overexpressed in COS cells was detected in both the resting and the inflamed peritoneal cavity (Fig. 5A). The fold increase in signal intensity in the inflamed exudate was

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** Kinetics of infection and peritoneal inflammation. A and C, and B and D, represent two experiments. CD1 mice were infected with 2500 (A and C) or 7500 (B and D) *T. gondii* tachyzoites. At the indicated times, the peritoneum was washed and the exudate assessed for total cells (A and B, ○) and total extracellular tachyzoites (C and D, filled bars). Cytospins were prepared and counted to determine total granulocytes (A and B, dashed line, □) and percentage of macrophages containing parasites (C and D, open bars; days 1–4 only). Each point represents a minimum of three animals ± SE.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** Expression of A1 mRNA during peritoneal toxoplasmosis. The same exudate samples were used as in Fig. 1, A and C. At the indicated days p.i., the cell suspension was extracted for total cellular RNA. Northern blots (4 μg/lane) were sequentially probed for A1, Bcl-2, and 28S rRNA. Each lane represents an individual mouse. P indicates P388D1 (4 μg), a cell line constitutively expressing A1 mRNA (37).

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** A1 expression is dose dependent and correlates with inflammation. BALB/c mice were infected with either $1 \times 10^3$ or $1 \times 10^4$ tachyzoites, as indicated. At the indicated days p.i., peritoneal cellularity was determined (open bars). RNA was prepared from total peritoneal exudate and assessed for A1 mRNA expression (filled bars) by sequentially probing Northern blots for A1 and 28S rRNA. The data are presented as fold increase relative to two control animals (dashed line). Error bars = SE (n = 3). *, $p < 0.05$ in comparison with $1 \times 10^3$, day 3. **, $p < 0.05$ in comparison with the corresponding dose at day 3.
FIGURE 4. Expression of A1 in irritant peritonitis. Peritoneal cells were obtained from BALB/c mice that were either untreated (0) or injected 6 days earlier with 1000 *T. gondii* tachyzoites (T. gondii), thioglycolate broth (TG), or vehicle (VEH). RNA (4 μg) was analyzed on Northern blots sequentially probed for A1 and 28S rRNA. Each lane represents an individual mouse.

3.7 ± 1.0 (SE) (n = 3). These signals were ablated by competition with the antigenic peptide (Fig. 5B).

The reactivity of the anti-peptide Ab with peritoneal cells from normal and infected mice is illustrated in Fig. 6. Normal resident peritoneal macrophages were uniformly weakly positive, showing a cytoplasmic stain that had both a diffuse and a punctate character, with some concentration of the punctate stain in the perinuclear region (Fig. 6, A and C). In contrast, inflammatory macrophages from infected mice were highly variable with regard to intensity of staining, and approximately half of the macrophages showed an intense focal accumulation of stain in a perinuclear location (Fig. 6D). There was no evident correlation between A1 immunostaining and the parasitized state of the cell, as the infected and uninfected macrophage populations each included strongly and weakly immunoreactive cells (Fig. 6F, arrowheads) in similar proportions (data not shown).

Most inflammatory granulocytes were stained strongly by the anti-peptide Ab (Fig. 6E). It is difficult to distinguish neutrophils and eosinophils with certainty among the immunoreactive granulocytes. However, A1-negative granulocytes were uniformly eosinophils by eosin staining (data not shown; see Fig. 7). Therefore, it can be concluded that inflammatory neutrophils are uniformly positive, whereas eosinophils are largely unstained.

**A1 is constitutively expressed in normal neutrophils**

Next, the reactivity of the 855AP Ab with normal peripheral blood leukocytes was assessed (Fig. 7). As with the inflammatory infiltrate, granulocytes were mostly positive, and negative granulocytes were in all cases eosinophils. The number of negative eosinophils was similar to the total number of these cells determined by differential counts (data not shown). Therefore, A1 expression appears to be a constitutive property of neutrophils but not of eosinophils. Blood monocytes showed only a weak stain similar to that observed in resident peritoneal macrophages, whereas lymphocytes were either negative or occasionally weakly positive (Fig. 7B, arrowheads). Similar results were obtained with mice housed under specific pathogen-free conditions (data not shown), indicating that constitutive A1 expression does not reflect an inflammatory process.

**The elevation of A1 mRNA in inflamed peritoneum represents induction in macrophages**

The constitutive expression of A1 in neutrophils raises a question regarding the earlier observation of increased A1 gene expression in *T. gondii*-elicited cells: is this elevation due to up-regulation of A1 gene expression or simply to the recruitment of large numbers of neutrophils already expressing A1? To address this question, we examined A1 mRNA expression in inflammatory exudates in which the abundance of neutrophils had been either increased or decreased by separation of the exudate on a density gradient. As shown in Fig. 8, depletion of up to two-thirds of the neutrophils in the exudate had little or no effect on the relative abundance of A1 mRNA. Conversely, fractions enriched for neutrophils showed no increase and perhaps a slight decrease in A1 mRNA relative abundance. Finally, total peripheral blood leukocytes, despite containing a substantial number of A1-positive neutrophils, showed only a very low relative abundance of A1 mRNA (Fig. 8), and this level of expression was not reduced by removal of 93% of neutrophils with a density gradient (data not shown). These data are consistent with previous reports of the low abundance of RNA in neutrophils (47, 48). The results indicate that, despite intense immunostaining, neutrophils contribute only a very minor proportion of the RNA analyzed on Northern blots and that the observed increase in A1 mRNA primarily reflects up-regulated expression in the macrophage lineage.

**Bax and A1 mRNA levels are correlated during inflammation**

The function of A1 potentially involves interaction with proapoptotic bcl2s. Yeast two-hybrid analysis has shown strong physical interaction between A1 and Bax (39, 40), a proapoptotic bcl2 expressed in neutrophils and macrophages (33, 34). We therefore asked whether Bax is expressed during the inflammatory response to *T. gondii* as shown in Fig. 9, inflammatory exudates exhibit similar up-regulation of A1 and Bax mRNAs. The kinetics of Bax up-regulation closely resemble those of A1 (data not shown). As with A1, Bax mRNA abundance is not reduced in neutrophil-depleted exudate, indicating that the up-regulation is likely to occur in macrophages (Fig. 9). In addition to the expected major 1-kb species, a minor, lower m.w. RNA was observed. The identity of this species is unknown.

**FIGURE 6.** A, Elevation of A1 protein in inflamed peritoneal exudate. Western blots were probed with the 855AP Ab against the A1 N terminus. COS, A1-transfected COS cells (0.5 μg); RPC, resident peritoneal cells from a representative normal BALB/c mouse (10 and 22 μg in A and B, respectively; different mice were used for A and B); PEC, peritoneal exudate cells from a representative BALB/c mouse infected 4 days previously with 1500 *T. gondii* tachyzoites. B, Two replicate blots prepared and probed in parallel. For one of these blots, the Ab was preincubated for 15 min with 200 μg/ml of the antigenic peptide.
A1 shows apparent alternative subcellular localization during apoptosis of inflammatory leukocytes

To assess the potential involvement of A1 in the regulation of apoptosis, we asked whether the expression of A1 was altered in apoptotic inflammatory cells. TUNEL-stained apoptotic macrophages and granulocytes were detectable in *T. gondii*-elicited exudates, although infrequently. Dual staining of exudates for TUNEL and A1 expression indicated that apoptotic macrophages and granulocytes occasionally contained high levels of A1 localized to the nucleoplasm, in contrast to nonapoptotic cells in which only cytoplasmic A1 was observed (Fig. 10). Some artifactual staining of apoptotic nuclei was observed with secondary Ab alone (Fig. 10E), but this was less intense than that produced by the 855AP Ab (Fig. 10B). In apoptotic macrophages with partial TUNEL staining, A1 was observed only in the cytoplasm (data not shown). Cytoplasmic A1 remained prominent in a portion of the apoptotic granulocytes and macrophages, so that in some cells a whole-cell staining pattern was observed (data not shown).

Discussion

The current study documents the occurrence of a protective Bcl-2 family member in inflammatory neutrophils and macrophages. The presence of A1 in these cell types is consistent with our previous studies of A1 expression in cultured cells. A1 mRNA was rapidly induced in macrophages by proinflammatory stimuli such as LPS or GM-CSF (37). A rapid, transient up-regulation of A1 protein by these stimuli has also been observed (49). A1 was also shown to be expressed in promyeloid 32D.3 cells induced to differentiate to neutrophils by granulocyte CSF (38). In the latter experiment, the appearance of A1 mRNA paralleled the appearance of mature neutrophils. However, it could not be determined whether this up-regulation of A1 resulted from the activating effects of G-CSF or was alternatively a concomitant of differentiation per se. The results described here indicate that at least the latter interpretation is correct: normal mature mouse neutrophils constitutively express high levels of A1.

While our manuscript was in preparation, two reports appeared describing the expression of A1 in neutrophils. Hatakeyama et al. (50) reported RT-PCR analysis demonstrating A1 expression in...
murine neutrophils. Chuang et al. (51) described the occurrence in human neutrophils of mRNA encoding A1 and a second protective
bcl2, McI-1. Consistent with our findings, human A1 mRNA was
costitutively expressed in neutrophils. In addition, neutrophil A1
mRNA was up-regulated 2–4-fold by G-CSF and by LPS. The
effect of these agents on mature murine neutrophils with respect to
A1 mRNA and protein levels remains to be examined.

The report of Hatakeyama et al. (50) describes the existence of
four A1 genes in the mouse, three of which encode full-length A1
proteins and are coexpressed in neutrophils. A1-a is the isoform
previously studied with respect to antiapoptotic function (38)
and against which the 855AP Ab was raised. The three isoforms are
96–97% identical overall but diverge within the N-terminal pep-
tide used to prepare the 855AP Ab. We are currently investigating
the isofom specificity of this Ab.

The increased expression of A1 mRNA elicited by T. gondii was
generally coordinate with the abrupt influx of inflammatory cells at
days 3–4 in infected mice. Consequently, the increase in A1 ex-
pression could reflect either recruitment of A1-expressing cells or
induction of A1 expression during or subsequent to extravasation.
Our results demonstrate that both of these processes occur: infil-
trating neutrophils constitutively express A1; yet the observed in-
crease in peritoneal cell A1 mRNA is primarily due to up-regula-
tion in macrophages. This conclusion is further supported by the
observation that in individual mice, A1 up-regulation is not always
precisely coordinate with increased peritoneal cellularity. For ex-
ample, of the three mice examined at day 3 in Fig. 2, only two
show elevation of A1 expression, yet all three are comparable with
respect to cellularity and frequency of neutrophils (data not
shown). This suggests not only that A1 expression is induced but
that this induction is subsequent to extravasation. The induction
does not appear to be triggered by parasitization of macrophages.
Nevertheless, we have observed that total peritoneal A1 mRNA is
substantially better correlated with the frequency of intracellular
parasitization than with peritoneal cellularity (data not shown),
suggesting that the signal(s) responsible for macrophage A1 ex-
pression is related to the pathogen-driven host response. Prelimi-
nary results show that mRNA for GM-CSF, a cytokine known to
induce A1 in macrophages, is up-regulated during T. gondii-elic-
ited inflammation and this up-regulation is coordinate with A1 ex-
pression: in the experiment shown in Fig. 2, with one exception
mice showed increased expression either of both genes or of nei-
ther gene (data not shown). The exception was an uninfected
mouse with basal A1 and elevated GM-CSF. Therefore, inflam-
atory cytokines such as GM-CSF are potential mediators of A1
up-regulation.

An interesting aspect of our results is that a portion of apoptotic
leukocytes express A1 and that in some apoptotic cells A1 displays
an unusual nucleoplasmic localization. Three possible (not mutu-
ally exclusive) hypotheses are suggested by these findings. The
first is that upon translocation from cytoplasm to nucleus, A1 no
longer functions to counteract apoptosis. The ability of A1 to
inhibit apoptosis in neutrophils was recently demonstrated using
gene targeted mice lacking the A1-a isoform (52). Studies in other
cell types have confirmed the anti-apoptotic activity of A1-a (38,
53) and A1-b (50). However, it is possible that the functionality of
A1 is affected by altered localization or by modifications in
apoptotic cells. Relocalization of A1 may thus represent part of a
proapoptotic program. Two other bcl2s, Bax and Bcl-XL, have
been shown to alter their subcellular localization (from cytosol to
membranes) upon induction of apoptosis (54). The functional
significance of such relocalization remains unclear. The nucleo-
plasmic localization of A1 is a novel feature of this Bcl-2 family
member that we have previously observed using overexpression
systems (R. D. Somogyi et al., manuscript in preparation). A
second hypothesis is that nuclear A1 retains antiapoptotic function
in inflammatory leukocytes but that apoptosis proceeds via an
A1-independent pathway in these cells. The existence of A1-inde-
pendent apoptotic pathways is supported by a recent study
demonstrating that induction of A1 in human monocytes

![FIGURE 9. Bax mRNA is up-regulated in parallel with A1. The Northern blots used for determination of A1 mRNA in Fig. 8 were reprobed with Bax cDNA. Lanes C, Individual uninfected mice; lanes Tot, each lane represents unseparated exudate from a pool of five mice at day 5 p.i.; lanes L, neutrophil-depleted interfaces from the same two pools. The major Bax species migrates at ~1.0 kb.](http://www.jimmunol.org/DownloadedFrom)
fense to *T. gondii* (60, 61) and that can induce apoptosis in macrophages (62, 63), has been reported to up-regulate Bax in this cell type (33). It will be of interest to determine the ability of A1 to counter the effects of nitric oxide and other potential apoptotic inducers.

Neutrophilic inflammation is often highly transient, yet in other cases, such as the peritonitis we have examined, it is maintained for many days. More sustained neutrophilic responses might result from prolonged expression of immigration signals or alternatively from delay of apoptosis, or both. Neutrophils harvested from certain inflammatory sites have been shown to have enhanced longevity in culture relative to normal peripheral blood neutrophils (10–12) (52), whereas the reverse has been reported for neutrophils elicited by thioglycolate broth (2). It is possible that these various inflammatory environments differ in their ability to maintain or enhance neutrophil A1 expression. Preliminary results indicate that A1-negative peritoneal neutrophils occur with high frequency within 8 h after thioglycolate injection (data not shown). Conversely, the enhanced longevity of proteose peptone-elicited neutrophils has been shown to be an A1-dependent phenomenon (52). The ability to examine the expression of the A1 protein in individual cells will facilitate investigation of the role of this molecule in the modulation of inflammatory reactions.

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**References**


**FIGURE 10.** Apoptotic inflammatory cells express A1 in the cell nucleus. BALB/c mice were infected with 2000 *T. gondii* tachyzoites. Exudates were harvested at day 5 p.i. from nine mice and pooled. In this experiment, day 5 p.i. corresponded to a stage of inflammation similar to day 4 p.i. in Figs. 1 and 2. Cytospin preparations were stained by TUNEL (green) and then immunostained either with 555AP (red) (A–C) or with secondary Ab alone (D–F). A, Nomarski optics combined with green fluorescence (grayscale image). M and G indicate, respectively, a macrophage and granulocyte with nuclear TUNEL staining. B, Same field viewed under red fluorescence. C, Same field viewed under combined red and green fluorescence. The yellow signal indicates colocalization of TUNEL labeling and A1. Arrows indicate TUNEL-negative cells containing cytoplasmic A1. D–F, A field viewed under, respectively, Nomarski optics, red fluorescence, and green fluorescence. The arrowhead indicates a TUNEL-positive cell. E, Representative background staining associated with apoptotic nuclei. Asterisks indicate staining associated with parasites that may be artifactual. The results are representative of two similar experiments. Original magnification, ×250.


