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

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*J Immunol* 1999; 163:412-419; 
http://www.jimmunol.org/content/163/1/412
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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

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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.
**T. gondii** was maintained on human fibroblasts as described previously (41). Supernatants containing 10^7 tachyzoites/ml were diluted in calcium- and magnesium-free PBS (PBS-CMF). A volume of 0.4 ml containing 1 × 10^6 to 10^7 tachyzoites was injected i.p. Vehicle controls used tissue culture medium diluted in PBS-CMF. For initiation of thioglycolate peritonitis, BALB/c mice were injected i.p. with 1.0 ml of sterile 3% thioglycolate broth (Difco, Detroit, MI) aged several months.

**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 either individual mice 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 underlain with Ficoll/sodium diatrizoate (density, 1.090) and centrifuged at 150 × g for 20 min, washed several times with PBS-A, brought to 10 M/L 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).

**Protein content of lysates was assessed by the Bradford assay. Samples were separated on 15% SDS-PAGE gels and transferred to Immobilon-P membranes (Millipore, Bedford, MA) by wet electrophoretic transfer (Bio-Rad, Richmond, CA) in 25 mM Tris, 190 mM glycine for 40 min at 100 V. The membranes were blocked with 5% dry milk + 0.1% Tween-20 in PBS (block solution) and probed with the 855AP Ab (1:100) in block solution for 1.5 h. The blot was washed 9 times in PBS-CMF + 0.5% Tween-20, incubated in horseradish peroxidase-conjugated donkey anti-rabbit antiserum (Amersham) (1:10,000 in block solution), and washed as before. Detection was by enhanced chemiluminescence (ECL) according to the supplier’s recommended protocol. Quantitation was by densitometry (Molecular Dynamics). The m.w. standards used here are prestained and therefore only approximate. By the use of unstained standards and mass spectrometry analysis of purified A1, we have determined that the m.w. of A1 agrees with the predicted value of 20 kDa (R. D. Somogyi et al., manuscript in preparation).

**Immunocytocytochemistry**

Fixed cytospin preparations were permeabilized in 0.2% Triton X-100 in PBS-CMF for 2 min. Slides were rinsed in PBS-CMF, and endogenous peroxidase was blocked by incubation in 1 mM sodium azide, 10 mM glucose, and 1 U/ml 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 cytospin 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 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 I3, N2.1, or G/R filters (Leica, Deerfield, IL). Images were scanned into Adobe Photoshop 5.0.

**Results**

**A1 mRNA levels are increased in the inflamed peritoneal cavity**

Intraperitoneal infection with tachyzoites of the virulent RH strain of *T. gondii* led to a vigorous inflammatory response characterized by an abrupt increase in both granulocyte and mononuclear cell numbers in the peritoneal cavity at 3–4 days postinfection (p.i.). The granulocytes were predominantly neutrophils, with a variable number of eosinophils (<20% total granulocytes (data not shown)). The mononuclear cells were primarily macrophages, with the proportion of lymphocytes varying between 10 and 30% (data not shown). The leukocyte influx was accompanied by a steady number of eosinophils (≤20% total granulocytes (data not shown)). 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).

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. In three similar time course experiments, the maximal increase in A1 expression ranged from 5- to 12-fold. Similar kinetics were obtained with either outbred CD1 or BALB/c mice (data not shown). The kinetics of A1 expression were dependent on the dose of the organism and generally correlated with inflammation as assessed by peritoneal cellularity (Fig. 3, but see Discussion).
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.** 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.** 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.** 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.
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.

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**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.

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**FIGURE 5.** 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 artificial 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, Mcl-1. Consistent with our findings, human A1 mRNA was constitutively 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 peptide used to prepare the 855AP Ab. We are currently investigating the isoform 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 expression 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: infiltrating neutrophils constitutively express A1, yet the observed increase in peritoneal cell A1 mRNA is primarily due to up-regulation 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 example, 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 expression is related to the pathogen-driven host response. Preliminary results show that mRNA for GM-CSF, a cytokine known to induce A1 in macrophages, is up-regulated during T. gondii-elicited inflammation and this up-regulation is coordinate with A1 expression: in the experiment shown in Fig. 2, with one exception mice showed increased expression either of both genes or of neither gene (data not shown). The exception was an uninfected mouse with basal A1 and elevated GM-CSF. Therefore, inflammatory 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 mutually 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 relocation remains unclear. The nucleoplasmic 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-independent apoptotic pathways is supported by a recent study demonstrating that induction of A1 in human monocytes...
correlates with selective resistance to a subset of apoptotic inducers (55). The third hypothesis is that the induction of A1 in apoptotic leukocytes (at least in macrophages, which do not uniformly express A1 before apoptosis) occurs after the onset of apoptosis. Recent studies have indicated that members of the TNF family that induce apoptosis can also up-regulate antiapoptotic activity dependent on protein synthesis (56–58). TNF-α in fact up-regulates A1 in human endothelial cells (59). However, neutrophil sensitivity to TNF-α-mediated apoptosis was not enhanced by A1 deficiency (52).

The occurrence of apoptotic macrophages implies the existence of inflammation-associated apoptotic inducers, because this cell type normally shows little spontaneous apoptosis. The presence of proapoptotic factors is also implied by the propensity of macrophages from T. gondii-infected mice to enter apoptosis upon culture (13). Our results indicate that Bax may serve as a mediator of the effects of such apoptotic inducers in inflammatory macrophages. Nitric oxide, a molecule that is associated with host defense 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.

Acknowledgments

We thank Dr. Yan Fen Ma for assisting with T. gondii culture. We gratefully acknowledge the technical assistance of Ms. Nicole Kawachi.

References


