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Serum deprivation induced apoptosis in macrophage is mediated by autocrine secretion of type I IFNs

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Apoptosis can be triggered by different forms of cellular stress. We here show that serum deprivation induces the expression and secretion of type I interferons and results in apoptosis in RAW 264.7 cell in a caspase dependent manner. Administration of either IFN-α or IFN-β antibody partially inhibits apoptosis while the two antibodies used together totally prevents RAW264.7 cell from apoptosis. GM-CSF, but not M-CSF and IL-3, protects serum deprivation induced apoptosis. Inhibition of JAKs also prevents macrophages from apoptosis. Activation of MAPKs is not required for serum deprivation induced apoptosis. Our results are the first to demonstrate that serum deprivation-induced apoptosis acts through autocrine secretion of type I interferons.

Keywords: apoptosis; autocrine secretion; IFNs; serum deprivation.

Introduction

Infiltrating of hematopoietic cells including lymphocytes, neutrophils and macrophages, into the target tissues is a response to inflammation. The intensity and duration of the inflammatory reaction is partly governed by apoptosis of the cells that carry out the attack.[1] Therefore, the regulation of apoptosis of the infiltrating cells is the key event that defines the initiation and the progression of inflammation. It remains uncertain whether the effects of immune cell apoptosis on inflammation are beneficial or harmful.

Macrophages are important in innate immunity because of their non-specific response to almost all infectious microorganisms. Macrophages are specialized phagocytes responsible for ingesting and digesting microorganisms. Activated macrophages produce cytokines such as tumor necrosis factor and interleukin-1, which are crucial mediators of host inflammatory responses.[2] As macrophages play a crucial role in immunity, the lifespan of activated macrophages is important in physiological and pathological processes. Understanding how the apoptosis of macrophages is controlled will help to develop methods to modulate the balance of inflammatory responses.

Unlike their precursors (monocytes), macrophages are long-lived cells when they are in a resting stage. It has been suggested that the lifespan of macrophages in inflammation sites was extended by survival factors such as apoptosis inhibitor factor and nerve growth factor secreted by macrophages or other cells.[3,4] Meanwhile, macrophage death has been observed in many inflammatory diseases.[5–7] Apoptosis is a process of cell death manifested by fragmented nuclei with condensed chromatin and shrunken cytoplasm.[8,9] Mitochondrial release of apoptogenic factors such as cytochrome *c* (cyt *c*) occurs in many apoptosis processes.[10] Cyt *c* release can be regulated by Bcl-2 family proteins,[10,11] by translocation of Nur77 or p53 to mitochondria,[12,13] and by caspase activation.[14] Macrophage apoptosis independent of the known caspases has been reported recently.[15] The pan-caspase inhibitor zVAD, which prevents apoptosis in many different types of cells including activated T cells and B cells, actually promotes apoptosis of LPS-activated macrophages. Apoptosis of immune cells including macrophages has been found to occur in inflammatory diseases.[5–7] Apoptosis of macrophages may serve to restrict and focus the immune response, but it may also reduce the host defenses against infectious pathogens or contribute to immune suppression. Although our understanding of apoptosis has greatly improved in recent years, apoptosis of macrophages is still poorly understood in comparison with the apoptosis of lymphocytes.[16–18] Apoptosis of macrophages could also result from autocrine secreted pro-apoptotic factors such as TNFα, nitric oxide, upon stimulation.[19–21]

Mitogen activated protein (MAP) kinases are signaling protein modules used by eukaryotic cells to transmit signals.[19–22] Several independent MAP kinase pathways have been identified in mammals including the extracellular regulatory protein kinase (ERK),[23] c-Jun N-terminal kinase (JNK)[23,24] and p38 MAP kinase pathway.[25,26] Activation of MAP kinase pathway has been shown to be involved in apoptosis of different cells, such as NGF-withdrawal induced PC12 death,[27] H_2O_2 stress induced cell death[28] and glucocorticoid induced T cell apoptosis.[29] The molecular

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mechanism mediating these events remains unclear. Apoptosis has been observed in many different cell types and is triggered by a variety of extracellular stimuli.[30,31] It is believed that apoptotic stimuli ultimately lead to the activation of a protease cascade that results in apoptosis.[11,32] Although a number of molecules that partake in the process of apoptosis have been identified, the mechanism by which apoptotic signal triggers the activation of the cascade has not been fully elucidated. The precise role of MAP kinase pathway, as well as the individual components of these pathways during an apoptotic process, needs more extensive study.

In this study, we report that serum deprivation induced apoptosis in macrophage is mediated by autocrine secreted type I IFNs. Serum deprivation in macrophage induces the expression and secretion of IFN-α and -β, which lead to apoptosis. Neutralization of IFNs with antibodies protects macrophage apoptosis induced by serum deprivation.

Materials and methods

Reagents: SB203580, SP600125, U0126 and z-VAD-FMK, S-Methyl-ITU were purchased from Alexis Biochemical. Anti-phosphorylation-ERK, JNK, p38 antibodies were from New England Biolabs, Inc. Propidium iodide (PI) and 2-aminopurine were the products from Sigma. Recombinant murine IL-3, M-CSF, GM-CSF were from Peprotech Inc.. Jak inhibitor I was from Calbiochem. Anti-mouse IFN-α, and -β polyclonal antibodies were from R&D.

Cell culture and treatments: RAW 264.7 cell was maintained in RPMI1640 medium supplemented with 10%FBS. For the induction of apoptosis, 5×10^5 cells/well were seeded into 12-well plate and cultured at 37°C for 18 h. Cells were washed once with serum-free RPMI1640 medium and re-cultured in RPMI1640 medium without serum in the presence or absence of other reagents for different time.

Flow cytometry assay: Cells were collected after treatment and washed 3 times by PBS, then incubated with PBS containing 100 μg/ml Propidium Iodide(PI), 1%Triton X-100, 100 U/ml RNase for 30 min at 37°C, followed by FACS analysis.

DNA ladder assays: Cells were cultured in RPMI1640 medium supplemented with 10%FBS. To induce apoptosis, cells were removed to RPMI1640 medium without FBS and cultured for 24 h. Cells were then collected and suspended in lysis buffer containing 10 mM Tris·Cl, pH8.0, 10 mM NaCl, 10 mM EDTA, 100 μg/ml proteinase K, 1%SDS, and incubated at 37°C for 4 h. Lysate was extracted with phenol:Choloroform (1:1,V:V) for 3 times. DNA was precipitated with 1/10 volume of 3 M sodium acetate (pH 4.0) and 2 volume of ethanol. The pellet was dissolved in 20 μl TE with 2 mg/ml RNaseA and subjected to electrophoresis on 1.5% agarose gel.

MAPK activity assays: Cell lysate in Laemmli reducing sample buffer was separated on 12% polyacrylamide gel. Total proteins were transferred onto PVDF membrane after electrophoresis. Western blot assay was performed using antiphosphorylated-ERK, JNK and p38 antibodies, respectively.

RT-PCR: Total RNA was isolated from cells by RNAgents (Premega). 4 μg total RNA was used for reverse transcription to generate first strand cDNA. It was then subjected to PCR with the primers listed in Table 1.

Nitric oxide assay: Cell medium was collected after treatment and mixed with equal volume of Greiss reagent.[33] Mixture was incubated at room temperature for 10 min. The absorbance of the reaction product was determined at 540 nm using spectrophotometer. Sodium nitrite was used as a standard.

ELISA for the secretion of type I interferon: Serum free mediums were collected and dried after RAW264.7 cells were cultured for different time, and coated into 96-well plate. The plate was blocked by PBS with 1%BSA and 0.1% Tween-20. IFNα or IFNβ antibody was added and incubated at 37°C for 1 h. AP-linked anti-rabbit IgG antibody was added and incubated at 37°C for anther hour. The pNPP as substrate was added after incubation. Immuno reaction was measured at A_{405} by Microplate Reader.

Results

Serum deprivation induced apoptosis is caspase dependent

Murine macrophage RAW264.7 cell underwent apoptosis following serum deprivation for 24 h (Figure 1A). Death cells were about 15% in total treated cells estimated by flow cytometry assay. Cell death was shown a typical apoptotic feature determined by DNA laddering assay (Figure 1B). To test weather macrophage cell death induced by serum deprivation is caspase dependent or independent, we pretreated the cells with pan-caspase inhibitor, zVAD followed by serum deprivation. It showed that zVAD prevented the cell from DNA laddering (Figure 1B), and from apoptosis as well (Figure 1C). It indicated that serum deprivation induced cell death is caspase dependent.

Activation of MAPKs is not required for apoptosis

MAP kinases have been identified to be involved in apoptosis of different cell types.[27–29] Therefore, we tried to address whether the macrophage apoptosis induced by serum deprivation required the activation of MAP kinases. Cell extracts were prepared from RAW264.7 treated by serum deprivation for different time. The assay for the activation of MAP kianses was performed by western blot using anti-phospho MAP kinase antibodies for ERK, JNK and p38, respectively. As shown in Figure 2A, all of these three kinases were activated. The activation patterns were similar. Initial activation started at 15 min and declined after 30 min.

Table 1. Gene specific primers for RT-PCR

Gene name		Primer sequence	Product size (bp)
IFN-alpha4	forward	gagttacactgcctttgcc	237
	reverse	attgagctgctgatggag	
IFN-beta	forward	gagttacactgcctttgcc	430
	reverse	gattcactaccagtcccaga	
IFN-gamma	forward	caagtggcatagatgtgga	923
	reverse	ttatttgtcattcgggtgt	
iNOs	forward	gagcgagttgtggattgttc	1380
	reverse	tgcttcagtcaggaggtttg	
TNF-alpha	forward	tcgtagcaaaccaccaag	417
	reverse	caatgactccaaagtagacc	
COX-2	forward	acctctgagatgctcttc	904
	reverse	acactctgttgtgctccc	
Bcl-2	forward	ggcatcttctccttccag	442
	reverse	ctacccagcctccgttat	
Bax	forward	agaggcagcggcagtgat	364
	reverse	aaagtagaagagggcaaccac	
p53	forward	gcagggtgtcacgcttct	773
	reverse	gataggtcggcggttcat	
beta-actin	forward	tgccgcatcctcttcctc	655
	reverse	cgccttcaccgttccagt	

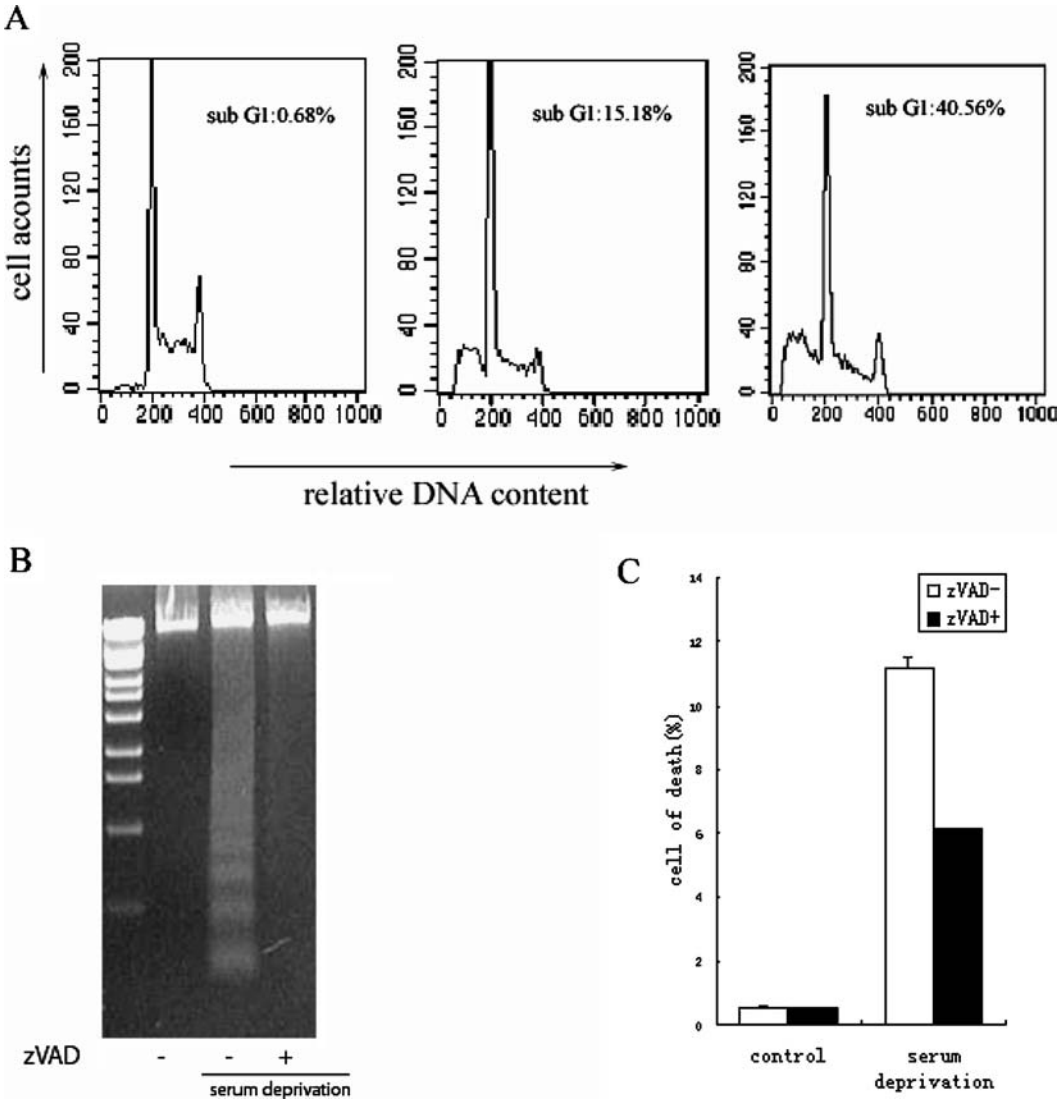
The second activation appeared at 8 h, and increased with time. In comparison with ERK and JNK activation pattern, p38 activation was relatively stable during the indicated time periods. We then tested if the activation of MAPKs was required for apoptosis. RAW264.7 cells were pretreated with MAPKs inhibitors, U0126, SP600125 and SB203580, respectively. The stress of serum deprivation was given for 24 h after the pretreatment. Cell death was analysed by FACS assay. As shown in Figure 2B, inhibition of each MAPK by their inhibitors did not prevent the apoptosis induced by serum deprivation. The result was confirmed by DNA ladder assay (Figure 2C). On the contrary, cell death was enhanced slightly by inhibitors of JNK and ERK. p38 inhibitor did not show any effect on apoptosis. It seemed that the inhibitors of MAPKs had a complicated effect on cell viability under the condition of serum deprivation.

Autocrine secretion factors are involved in serum deprivation induced apoptosis

Cell growth requires survival factors, most of which are growth factors and cytokines.[3,4] Serum deprived apoptosis may result from the absence of growth factors and cytokines. We used several factors including GM-CSF, M-CSF and IL-3 to test the effect on the protection of apoptosis induced by serum deprivation. It showed that GM-CSF, but not M-CSF and IL-3, partially protected apoptosis (Figure 3A)

Macrophage is able to secret a variety of cytokines that can trigger apoptotic signaling upon stimuli. Therefore, we tried to address whether the secretion factors were involved in RAW264.7 apoptotic signaling. Cells were cultured for 24 h in the medium without serum. The medium was then collected and used to culture fresh cells for 24 h. Apoptosis was measured by cell flow cytometry. As shown in Figure 3B -4, apoptosis was enhanced in used serum free medium. Death cells induced by Serum deprivation were about 10% of total treated cells. The death population was increased to more than 40% when the serum free medium was used again to culture fresh cells. As a control, the normal serum-containing medium did not have the effect on cell death when it was used again for fresh cells (Figure 3B-3). It suggested that autocrine secreted factors from RAW264.7 cells cultured in serum deprived medium contributed to the apoptotic signaling. Besides, autocrine secretion factors also showed an effect on cell cycle. They caused cells arrested at S phase when cells were cultured in used serum free medium with additional serum (Figure 3B-5). We next checked in serum deprived macrophage several possible secretion factors and their related genes, including TNFα, iNOS, IFN-α, -β, -γ, COX-2 and nitric oxide(NO). It showed that IFN-α, β and iNOS were remarkably induced while TNFα and COX-2 remained unchanged (Figure 3C). IFN-γ was not detectable (data not shown). Induction of iNOS implicates the induction of the production of NO. We measured the change of NO in serum free medium during the culture for 24 h. NO was increased with time (Figure 4A). Since a series

Figure 1. Induction and inhibition of RAW264.7 cell apoptosis. A. Cells were cultured in the medium with 10% or without serum for 24 h. Apoptosis was analyzed by FACS assay. B and C. Cells were cultured in the medium without serum in the presence or absence of zVAD for 24 h. Genomic DNA was extracted and resolved on 1.5% agarose gel (B). Apoptosis was measured by FACS analysis. The bar represents relative number of death cells (C). The results show the means ± SE ($n = 3$–4).

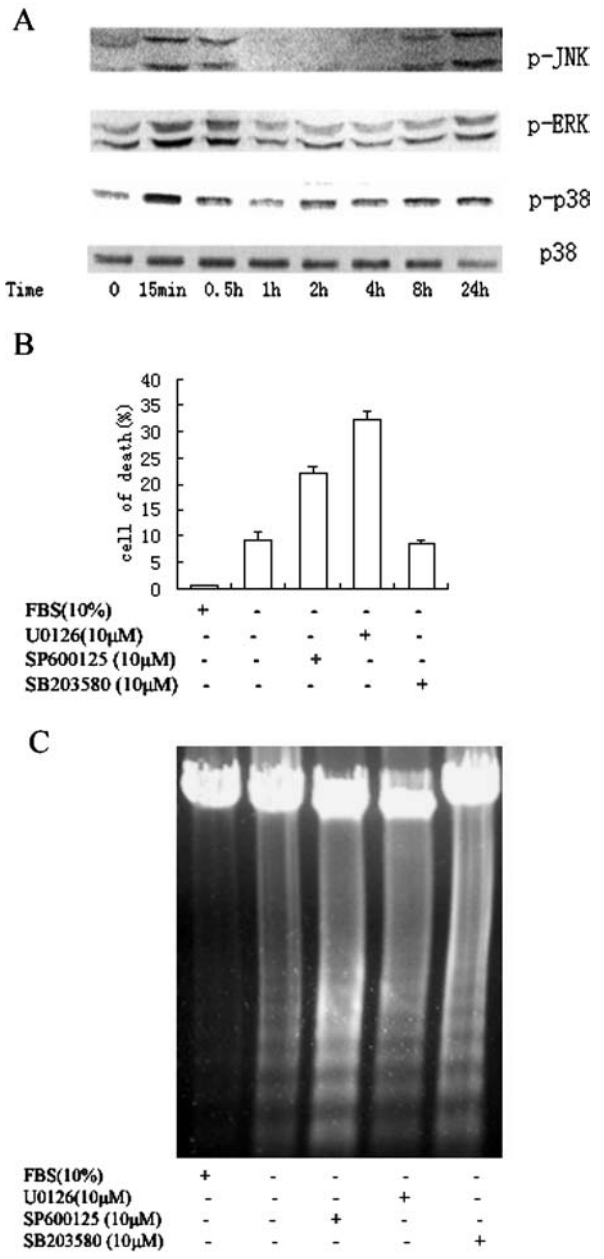


of apoptotic genes were induced upon different stimuli. We checked several genes in macrophage under the condition of serum deprivation. As shown in Figure 3C, Bax, Bcl2 and p53 remained the same.

Autocrine secretion of type I IFNs, but not NO, is involved in serum deprivation induced apoptosis

NO has been reported to induce apoptosis in several cell lines.[21,34] Induction of NO in macrophage under the condition of serum deprivation prompts us to address the effect of NO on apoptosis. We pretreated cells with iNOS specific inhibitor, S-Methyl-ITU. Apoptosis was measured by flow cytometry assay after cells were cultured for 24 h in the medium without serum. It showed that iNOS inhibitor did not have an effect on the protection of cell death (Figure 4A and B), indicating that NO does not contribute to the apoptotic signaling in this condition. We then tested if type I IFN was involved in this process. RAW264.7 cells were cultured in the medium without serum in the presence or absence of IFN-α or -β antibody, or with both antibodies. Cells were collected at different time within 24 h. Gene expression and cell apoptosis were measured after the treatments. As shown in Figure 5, death cell was increased (Figure 5A) with the increased expression (Figure 5B) and secretion of IFN-α and IFN-β (Figure 5C, left panel). The induction of IFNs did not appear in the cells cultured with serum (Figure 5C, right panel). Apoptosis appeared 12 h after serum deprivation and increased up to 24 h. Induction

Figure 2. Involvement of MAPKs in RAW264.7 cell during serum deprivation. A. Cells were cultured in serum free medium for different time as indicated. Total proteins were extracted, respectively and resolved on SDS-PAGE followed by western blot assay using anti-phospho JNK, ERK and p38 antibodies. B and C. Cells were pretreated with SP600125, U0126 and SB203580 for 30 min, respectively, and then cultured in serum-free medium for12 h. Apoptosis was measured by FACS. The bar represents relative number of death cells. The results show the means ± SE (n = 3–4) (B). Genomic DNA was isolated and resolved on 1.5% agarose gel (C).



of type I interferons was observed before 12 h, and increased with time up to 24 h after serum deprivation. Death cell was reduced by 20–30% in the presence of IFN-β antibody (Figure 5D). Protection effect was more remarkable in the presence of IFN-α antibody (Figure 5D). More than 80% of cells were protected. Apoptosis was almost totally blocked in the presence of IFN-α and -β antibodies. Inhibition of apoptosis was in a dose dependent manner. To confirm the effect of type I interferon on apoptosis, we added IFN-α or IFN-β or both in the medium with or without serum. Apoptosis was measured after 24 h culture. The addition of type I interferon did not induce apoptosis of RAW264.7 in serum medium. However, it greatly enhanced apoptosis in serum free medium. This effect appeared in a dose dependent manner (Figure 5D). We concluded that macrophage apoptosis induced by serum deprivation resulted from autocrine secretion of type I IFNs.

Inhibition of JAK prevents cells from apoptosis

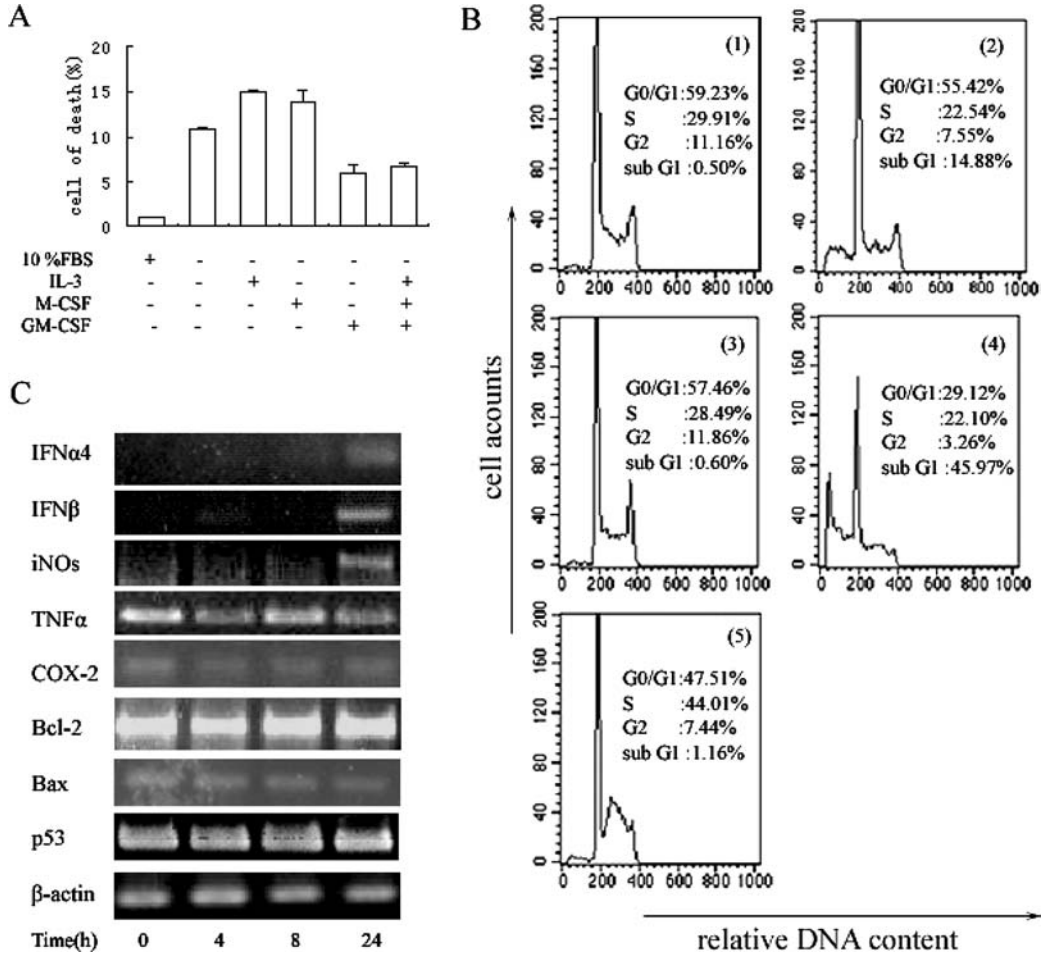
Double stranded RNA activated protein kinase (PKR) is one of the interferon inducible genes and plays a key role in macrophage apoptosis.[35,36] INFs trigger a signaling through JAK pathway. Therefore, we tested if inhibition of related pathway could prevent cells from apoptosis induced by autocrine secretion of type I interferon in serum deprived medium. JAK and PKR inhibitors were added to the serum deprived medium, respectively. Cells were cultured for 24 h. Apoptosis was measured by flow cytometry. As shown in Figure 5, PKR inhibitor did not show the protection (Figure 6A) while JAK inhibitor prevented apoptosis in a dose dependent manner (Figure 6B).

Discussion

Serum deprivation induced apoptosis has been studied in many cell types. However, the mechanism that induces the cell death under the condition of serum deprivation is still unknown. In immune cells, apoptosis occurs usually through a mechanism called AICD (activation-induced cell death).[15,37,38] Activated macrophages undergo apoptosis when they are exposed to cytokines, such as IFN-γ and –α.[37,39] We show here that serum deprivation induced macrophage apoptosis seems to take AICD mechanism. Serum deprivation supplies an environment to activate RAW264.7. Activated cells then become sensitive to autocrine secretion factors and lead to cell death (Figure 3B). Autocrine secretion factors do not cause apoptosis of macrophage in resting stage. They only induce a termination of cell cycle in G2 stage (Figure 3B-5). It indicates that serum deprivation from RAW.264.7 cell triggers two cascades, the induction and secretion of INFs, and the activation of the cells, which leads to the cells susceptible to the autocrine secretion factors.

Autocrine secretion cytokine can lead to apoptosis in macrophage treated by LPS.[20] We address whether autocrine secretion factors are involved in serum deprivation induced apoptosis in macrophage. Serum free medium has been used twice to test this notion. It shows that apoptosis is enhanced when cells are cultured in used serum free medium

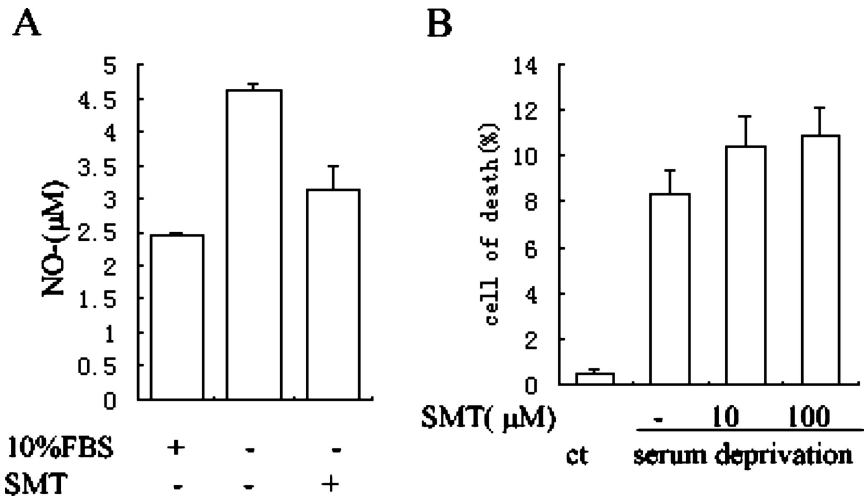
Figure 3. Involvement of autocrine secretion factors and growth factors in serum deprivation induced apoptosis in RAW264.7 cell. A.IL-3, M-CSF and GM-CSF (100 ng/ml of each) were added to the medium for 24 h, respectively, when cells were cultured in the medium without serum. Apoptosis was analyzed by FACS. The bar represents the relative number of death cells. B. Apoptosis assay by FACS for cells (1) cultured in the medium with serum for 24 h; (2) cultured in the medium without serum for 24 h; (3) cultured for 24 h in serum medium that has been used previously for 24 h; (4) cultured for 24 h in serum free medium that has been used previously for 24 h; (5) cultured in used serum free medium with additional serum for 24 h. C. Cells were cultured in serum free medium for different times as indicated. Total RNA was isolated and RT-PCR was performed using the primers listed in Table 1.



(Figure 3B), indicating that autocrine secretion factors are involved in apoptosis. We then check the changes of cytokine genes including TNFα, iNOS, IFN-α, -β, -γ, COX-2 and nitric oxide(NO). Serum deprivation does not cause the changes of TNFα and COX-2 gene. However, it induces the expression of type I interferon (Figure 3C). Induction of both IFN-α and IFN-β is increased with time (Figure 5B). It is correlated with the increase of apoptosis (Figure 5C). It also induces the expression of iNOS gene and the production of NO (Figure 3D and Figure 4A). IFN-γ is not detectable at basal level and after serum deprivation (data not shown). It is likely that RAW264.7 cell does not express type II interferon.

To test which cytokines involved in apoptotic process, we first evaluate the effect of NO on apoptosis with iNOS inhibitor. The data shows that inhibition of iNOS dose not have an effect on apoptosis (Figure 4). We conclude that NO is not the cause of apoptosis in serum deprivation condition. We than test the role of IFNs in serum deprivation induced apoptosis with antibodies. It is surprising that autocrine secretion of type I interferons plays a key role in serum deprivation induced apoptosis. IFN-α seems to have a more potent effect on apoptosis than IFN-β. Addition of IFN-α antibody to serum deprived medium reduces cell death by about 80% while IFN-β antibody reduces cell death only by 20–30% (Figure 5). However, application of IFN-α and -β antibody almost totally inhibits cell death. These data clearly demonstrate that autocrine secretion of type I interferon triggers an apoptotic signaling when the macrophage is cultured in the medium without serum. The role of type I interferon in apoptotic process is further tested by the experiments using JAK inhibitor, JAK inhibitor I. It has been known that JAK mediates interferon signaling. Inhibition of JAK by its inhibitor could block

Figure 4. Autocrine secretion NO is not required for serum deprivation induced apoptosis of RAW264.7 cell. A. Cells were cultured under the condition of serum deprivation for 24 h with or without iNOs inhibitor, SMT(100 μM). The amount of secreted NO^- was quantified according to materials and methods. B. Cells were cultured in serum free medium with or without SMT as indicated. Apoptosis was measured by FACS. The bar represents relative number of death cells. The results show the means ± SE ($n = 3$–4).



interferon signaling.[40,41] Our data show that JAK inhibitor only partially inhibits apoptosis although the inhibition is in a dose dependent manner (Figure 6B). It is likely that other pathway mediated interferon apoptotic signaling in macrophage under the condition of serum deprivation may also contribute to the process. 2-aminopurine, the PKR inhibitor does not have an effect on interferon mediated death signaling (Figure 6A), although PKR plays a key role in mediating interferon signaling and apoptosis.[35,36] It indicates that PKR may not be involved in interferon signaling under the condition of serum deprivation. It has been reported that autocrine secretion of TNFα and NO induce cell death in macrophage cells treated by LPS.[20] TNFα in RAW264.7 cell under the condition of serum deprivation is not changed (Figure 3). NO and iNOS are induced in response to serum deprivation. However, they are not involved in serum deprivation induced apoptosis. It indicates that autocrine secretion of cytokines in macrophage under different stresses may trigger apoptotic signaling by different ways.

Cell growth is dependent on survival factors that could be different growth factors and cytokines. Different cell lines may require different factors for surviving. It has not been known what factor is critical for RAW264.7 cell to survive. Chin *et al.* have demonstrated that TGF-β rescues serum deprivation-induced apoptosis via mitogen-activated protein kinase (MAPK) pathway in macrophage.[42] Our results show that GM-CSF, not M-CSF and IL-3, is essential for RAW264.7 surviving (Figure 3A). GM-CSF prevents macrophage from apoptosis. However, the survival of RAW264.7 seems to require more factors other than GM-CSF alone, because it prevents cell death by 40–50%. MAPKs seem not to be involved in serum deprivation induced apoptosis in our study. Although the activation of JNK, ERK and p38 is observed in the condition of serum deprivation, none of the MAPK inhibitors has the effect on the protection of apoptosis (Figure 2A). On the contrary, all MAPK inhibitors used exert some toxicity to cells under the condition of serum deprivation. Apoptosis of macrophage seems to be enhanced to different extent by the treatment of the MAPK inhibitors that are usually not toxic in the range of given concentration (Figure 2B and C). The data that inhibition of ERK activity by its specific inhibitor enhances apoptosis is consistent to the report by Chin *et al.* who show that activation of ERK by TGF-β rescues serum deprivation-induced apoptosis in RAW264.7.[42] It indicates that activation of ERK has a role in anti-apoptosis under the condition of serum deprivation. We observe a repeated activation of MAPKs during serum deprivation for 24 h. Initial activation appears fast and declined in half an hour. The activation restarts at several hours later and lasts relative long (Figure 2A). ERK pathway should be involved in the early stage in serum deprivation induced apoptosis, since the effect of TGF-β on the activation of ERK occurs in early time.[42] We have measured the activation of MAPKs within a relative long time, from 0 to 24 h while Chin *et al.* only check the activation of MAPKs in early stage in serum free condition. JNK signaling cascade has also been implicated in the regulation of apoptosis in a variety of mammalian cell types.[27–29,43] Zhang *et al.* have shown that JNK and other signaling molecules including p53, Akt, NF-kappa B, ERK, JNK, p38, PKC and IFN-gamma are involved in oxidative stress induced cell death.[43] An interactive pathway involved in cellular response to oxidative stress is proposed. With this regard, the involvement of MAPKs in serum deprivation induced apoptosis could be complicated. Nevertheless, macrophage under the condition of serum deprivation may be very sensitive to MAPK inhibitors or other chemical

Figure 5. Involvement of autocrine secretion of type I IFNs in serum deprivation induced apoptosis of RAW264.7 cell. Cells were cultured under the condition of serum deprivation for different times as indicated. A. Apoptosis was analyzed by FACS. The bar represents relative number of death cells. B. The induction of IFN-α and -β was analyzed by RT-PCR C. The secretion of IFN-α and -β was analyzed by ELISA. The bar represents the quantity of secreted IFN-α or -β. D. Cells were cultured under the condition of serum deprivation for 16 h with or without antibody as indicated. Apoptosis was analyzed by FACS. The bar represents relative number of death cells. E. Cells were cultured in the medium with or without serum for 24 h in the presence of IFN-α and -β, respectively. Apoptosis was analyzed by FACS. The bar represents relative number of death cells. The results show the means $\pm$ SE ($n = 3$–4).

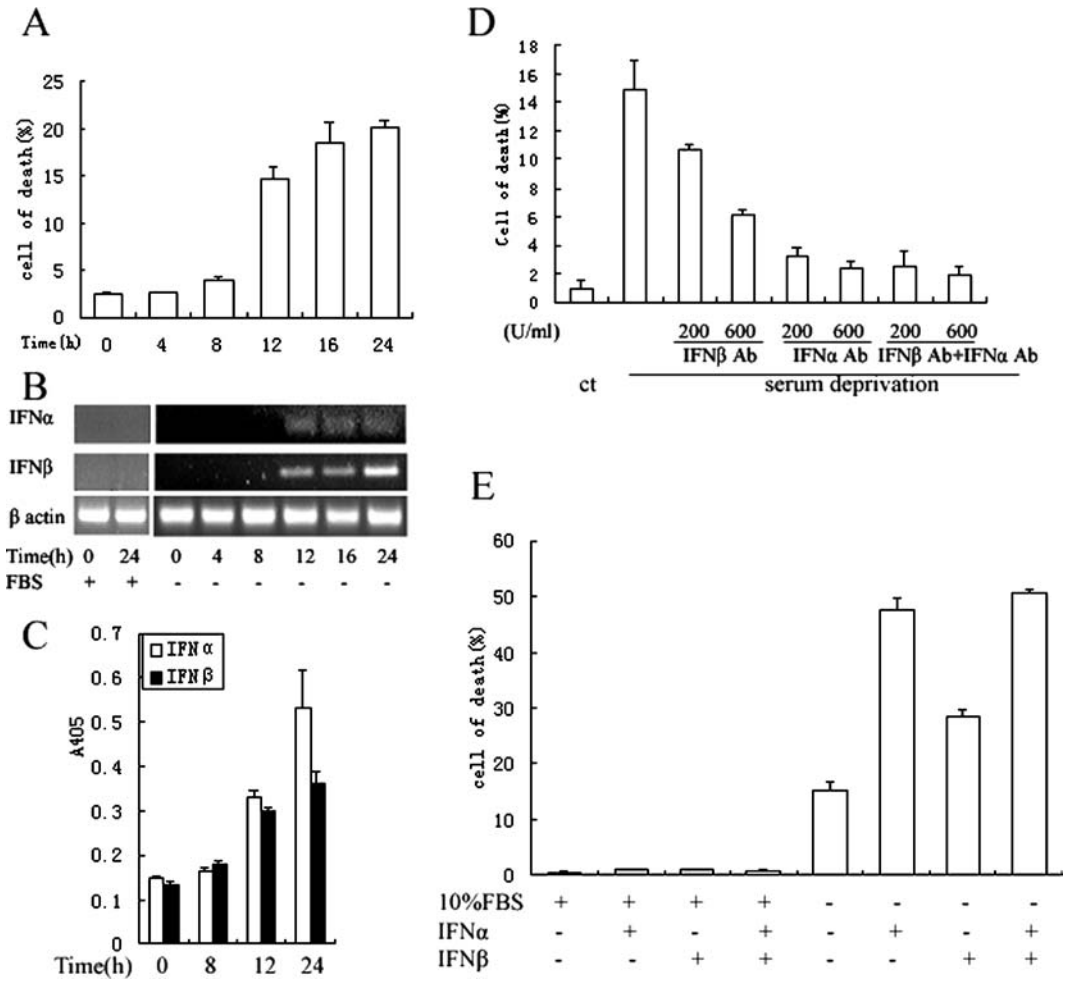
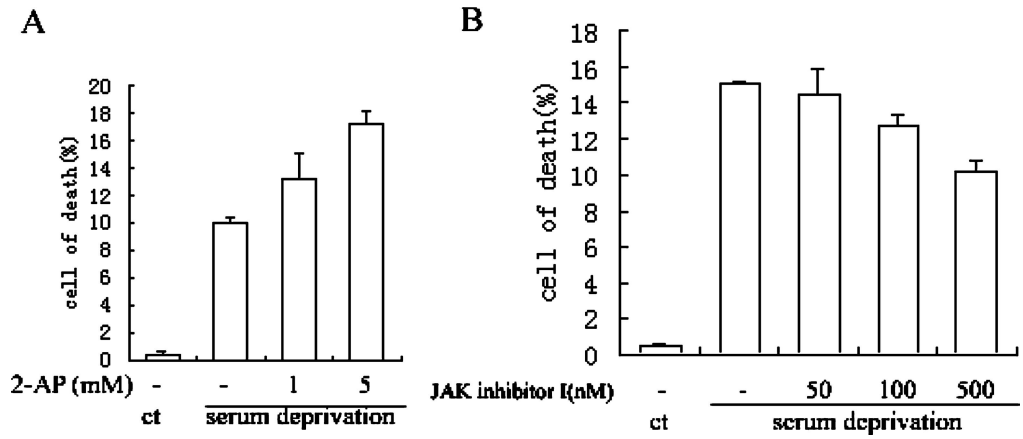


Figure 6. Involvement of JAK and PKR in serum deprivation induced apoptosis in RAW264.7 cell. Cells were cultured in serum-free medium containing different amount of 2-AP(A) or JAK inhibitor I (B) for 16 h. Apoptosis was analyzed by FACS. The bar represents relative number of death cells. The results show the means $\pm$ SE ($n = 3$–4).



compounds. Thus, the conclusion should be cautious from the experiments alike.

Serum deprivation induced apoptosis has been investigated for many years. The mechanism for this event has not been well addressed. Our results first demonstrate that serum deprivation induced apoptosis in macrophage is triggered by autocrine secretion of type I interferon. How does type I interferon trigger the apoptotic signaling under the condition of serum deprivation remains uncertain, which needs further investigation.

Acknowledgments

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