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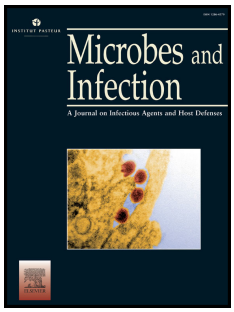

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Opinion article

Apoptosis in infection

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Abstract

Apoptosis is one of the principal responses that human cells have at their disposal when faced with changes in their environment. Microbial infection is a massive challenge to a cell, and it is unsurprising that the apoptosis apparatus has been implicated in numerous infections. However, looking at the available data, the impression is one of bewildering complexity. Microbial proteins and other molecules that are often poorly understood interact, with uncertain specificity, with host cell components of varying function, triggering signaling pathways that are ambiguously linked to the apoptotic machinery. Accordingly, many pathogens have been found in different studies both to induce and to inhibit apoptosis. I will here try to present some of the principles of apoptosis and of infection, and to provide a viewpoint on the question how the two are linked. I will further give the reasons for my personal opinion that apoptosis-induction is in most infections beneficial to the host.

Keywords: Apoptosis; infection; immune response

1. Introduction

Much has been written on the issue of apoptosis and infections. Many beautiful studies have generated data, investigating how apoptosis may contribute to the outcome of an infection. Numerous well-researched review articles have collated and presented the results from these studies. It has however proved impossible to simplify the role of apoptosis in infections into a picture with a few broad strokes. This may indeed not be possible. Agents of infectious disease are so diverse that they generate conditions only very loosely similar to each other. Apart from the presence of a second organism in the system that tries to replicate and whose replication has to be stopped, there may be very little that is common to the infection by two different pathogens. A Herpes virus may replicate in the nucleus of epithelial cells and persist in neurons. *Mycobacterium tuberculosis* may enter macrophages and reprogram them to suit its own purposes. The molecular reaction may therefore not overlap in too many respects.

On the other side, the apoptosis system has many features that increase the potential complexity. Although there is one core signalling pathway of apoptosis, involving mitochondria, and one less often used pathway originating from cell surface molecules, the apoptosis system can be triggered by many stimuli that a cell receives. This requires that many signalling pathways, which are activated in response to these triggers, are somehow molecularly linked to the apoptosis pathway. Besides such pro-apoptotic pathways, many signalling events can be anti-apoptotic and may mitigate pro-apoptotic signalling. During infection, various signalling pathways respond, and they may have both pro- and anti-apoptotic signalling qualities. If we try to summarize the available data, we can only create a maze of triggers, inhibitors and signals.

Can we still make any sense of the whole, or should we confine our efforts to the close investigation of one pathogen and one situation of infection at a time? I will here try to argue that apoptosis is a defence mechanism that serves a purpose by actual cell death in some situations and perhaps by cell activation in other cases. I will not focus on the actual interaction of microbes with a host cell and limit this to a few examples. Rather, I will discuss

some of the principles of apoptosis, of its general purpose and of the outcomes of apoptosis, in the attempt to explain how I have arrived at this opinion.

2. Apoptosis: general principles

Cell death by apoptosis is a common event in mammals. When a cell dies by apoptosis, it does so in consequence of the activation of a dedicated pathway. Components of the apoptosis apparatus are found in simple organisms, and it is generally assumed that the apoptosis apparatus – at least with some core components – evolved early on and was later put to a number of uses in more complex organisms [1].

In complex animals, such use has been documented in particular in three areas, not necessarily very clearly separated. One area is developmental biology: apoptosis is initiated in cells that are no longer needed and that may hinder further development. Famous examples include the tadpole shedding its tail, the human embryo deleting the webbing between its fingers or the shaping of the developing brain. The second area is homeostasis in proliferating tissues: neutrophil granulocytes, for instance, are constantly produced in the bone marrow and released into the peripheral blood, where they die after a few hours by apoptosis.

The third area is more complex, and it is here where contact with microbes comes in. This area of apoptosis encompasses those situations where the body has to react to certain stimuli, and where individual cells then may make the decision to die. Here apoptosis can often be regarded as a defence reaction. Chemicals, physical insults, irradiation may be dangerous: cells may be permanently damaged and may become unfit to live; they may acquire genomic mutations that make them actually dangerous. Killing off such cells, or having them die, would be a useful employment of the apoptosis system. Cells further may become infected by microbes and serve as a basis for microbial replication. Getting rid of infected cells, if possible including the microbe, will be helpful to the body [1, 2].

3. The mechanics of apoptosis

We now know much about the apoptosis pathway. There is however also much we do not know. Large gaps in our knowledge especially exist in the question of how a cell actually makes a decision to die or to survive. Sometimes an apoptotic stimulus may kill one particular type of cell but not another. In an experimental situation, a stimulus may kill 50 % of the cell population but not the other half – why would that be? Which qualitative, which quantitative differences are at work? This is highly relevant to microbial infections, where we often see weak induction of apoptosis, frequently detectable only in a small proportion of the cells exposed.

The regular execution of the apoptotic pathway always involves the activation of effector caspases, to the degree that apoptosis is often operationally and experimentally defined by caspase-activity. Caspase-3 is the main effector caspase [3, 4]. Caspase-3 is activated by one of two initiator caspases. These initiator caspases themselves can be activated by two separate upstream signalling pathways, known as the extrinsic or death-receptor mediated pathway, and the intrinsic or mitochondrial pathway. In the extrinsic pathway, membrane-spanning receptors such as CD95 (Fas), tumour necrosis factor receptor 1 (TNFR1) or Toll-like receptor 3 (TLR3) recruit signalling platforms that can in some situations activate the initiator caspase-8. In the mitochondrial pathway, cytochrome *c* is released from its location in the space between the two mitochondrial membranes into the cytosol, where it binds to Apaf-1, which then oligomerizes and activates the initiator caspase-9. As mentioned above, active caspase-8 and -9 then activate caspase-3 [5].

The release of cytochrome *c* is regulated by the Bcl-2-family of proteins. The about 15 recognised members (there is uncertainty regarding some members) interact among themselves, some are pro-, others anti-apoptotic, and their interaction determines whether cytochrome *c* is released. The release itself is now fairly clear: two pro-apoptotic Bcl-2-family proteins, Bax and Bak, can form pores in the outer mitochondrial membrane (this has been observed microscopically for Bax [6, 7]) and release cytochrome *c* directly. Bax/Bak are thus

the effectors of mitochondrial apoptosis, and regulation of their activation is the purpose of the other Bcl-2-proteins. Bax/Bak are activated by direct interaction with one of at least three other pro-apoptotic Bcl-2-family proteins (called Bim, (t)Bid and Puma). The anti-apoptotic Bcl-2-family proteins, Bcl-2, Bcl-X_L, Bcl-w, Mcl-1 and A1/Bfl-1, bind the pro-apoptotic proteins and inhibit them directly [8].

This regulation of mitochondrial apoptosis seems to be difficult to comprehend. As a general idea, the interaction of pro- and anti-apoptotic Bcl-2-proteins does determine apoptosis by regulating Bax and Bak, but with the existence of so many different proteins, many possible pairings, difficult-to-control levels of proteins and so on, many aspects remain undefined. Mice deficient in individual genes, often also deficient in combinations of two or more genes, have been made, and recently specific inhibitors of the anti-apoptotic proteins Bcl-2, Bcl-X_L and Mcl-1 have been introduced; these small molecules show great promise for tumour therapy. We know the basics and we have the instruments to understand it better, but we are still away from a comprehensive understanding of mitochondrial apoptosis.

Perhaps the most challenging part is to understand how the system is triggered, and this question will also be central to the considerations of apoptosis during microbial infections. We often have a stimulus that efficiently induces apoptosis but we do not know how. Take staurosporine, one of the most commonly used positive controls in apoptosis research. Staurosporine induces apoptosis through the mitochondrial pathway. We know that since over-expression of anti-apoptotic Bcl-2-family members or the combined loss of Bax and Bak protects against staurosporine-induced caspase-activation. We do not know however how Bax/Bak are activated by staurosporine-treatment. It may, to choose a random example, occur through the combined activation of pro-apoptotic Bim and Puma, together with a loss of Bcl-w, or through post-translational modification of Bcl-2-family proteins, or by many other molecular events.

There are certainly simple examples where we can identify pro-apoptotic pathways from the stimulus to a cellular receptor, to the requirement or participation of individual Bcl-2 proteins

in apoptosis induction. There are however substantial gaps in our knowledge how many pro-apoptotic stimuli are channelled into the core system of mitochondrial apoptosis.

Thus, although we understand the principles of mitochondrial apoptosis, its use by a human cell is often beyond complete comprehension. A human cell can respond to many situations – changing the conditions of signal input, physical or chemical insults, sometimes infections – by initiating apoptosis. This requires that the apoptotic pathway is linked to many other signalling pathways. During an infection the activity of many signalling pathways will be altered. Most likely, some will be tuned towards a pro-, others towards an anti-apoptotic effect. To predict the outcome, especially on the basis of the poor quantitative data we have in most situations, is exceedingly difficult.

4. Microbes and apoptosis: general concepts

A huge number of chemicals and other insults to a cell have been found to induce apoptosis. The interpretation seems justified that all strong disturbances of a cell's environment can be stimuli to induce apoptosis although cells will differ substantially from each other, especially depending on their differentiation status. Immature lymphocytes or neutrophil granulocytes for instance die with little encouragement, fibroblasts are much sturdier and so on.

The contact with microbial agents, very generally speaking, often is a tremendous challenge to a cell and a major disturbance of its life. When we look at it from this angle we do expect that the apoptosis system is triggered by infection. Perhaps it is even surprising how infrequent clear apoptosis is as a response to infection.

One of the striking features of microbes is how different they can be from each other. Bacteria for instance cover such a wide biological spectrum that, in many respects, it makes hardly sense to speak of 'the bacterium'. Within this spectrum, we mostly investigate microbial agents that have evolved to engage in some sort of physiological, or perhaps pathophysiological interaction with human cells. Of the overwhelming diversity of microbes found on earth, only few are specialised to live on the human body, fewer even to cause

disease. With their very different biological features, viruses, bacteria, fungi and (protozoan or metazoan) parasites again have to be viewed separately.

If a virus has too strong a pro-apoptotic potential it will remove its own chance of replicating in an infected cell: we will never find such a virus since it will disappear quickly. To be a successful agent of disease, the virus will have to find a balance that permits its temporary replication before the infection is, as will be the case almost invariably, terminated by the immune system.

The large group of commensal bacteria, almost by definition, do not induce damage (such as by apoptosis) to the body. Commensals are therefore unlikely to cause much of an apoptotic response. What about pathogenic bacteria? When bacteria cause disease this is typically linked to closer interaction with human cells. Pathogenic bacteria may sometimes enter human cells, or they may damage epithelial layers, or they may stimulate host cells to an extent as to cause inflammation. In all these cases of strong interaction, apoptosis may play a role.

Finally, apoptosis induction is not necessarily coupled to the immediate infection of a cell, or to direct contact of a cell to the microbe. Rather, the immune system plays major roles in apoptosis-induction and sensitivity. Cytokines and chemokines in particular can have numerous and multi-faceted effects. Take tumour necrosis factor (TNF) and type I interferon (IFN I), two cytokines that are produced promiscuously during an infection. TNF has only weak pro-apoptotic activity in most physiological circumstances but it can experimentally induce apoptosis. Although probably of limited relevance *in vivo*, the pro-apoptotic activity of TNF is in fact one of the molecularly best-understood apoptosis signals. IFN I has in some situations been found to be an important pro-apoptotic stimulus [9]. In an intact organism, the immune system, from initial inflammatory mechanisms to specific T cell responses, will contribute to apoptosis and apoptosis-sensitivity during infection.

5. Apoptosis during viral infections

Virus-induced apoptosis has been covered by a number of review articles over the years (see for instance [10, 11]). For viruses it seems straightforward that apoptosis is not a desirable event. A virus depends on the cell's integrity to replicate. Perhaps is the first report on this contingency still the most impressive. Lois Miller's group noticed that an insect virus that had lost one particular gene (p35, which later turned out to be an inhibitor of caspases) induced apoptosis in cells in culture, resulting in many fewer viral particles produced [12]. Thus, the virus has pro-apoptotic activity, which is however normally not apparent.

This theme has been tested over and again. For instance, two groups of large DNA viruses infecting mammalian cells, Poxviruses and Herpesviruses, typically carry genes whose products look and appear to act like mammalian anti-apoptotic Bcl-2 proteins [10, 11]. Viruses typically pick up host cell genes and modify them for their purpose, so taking Bcl-2 and adapting it is an obvious way of blocking apoptosis. Poxviruses even seem to have modified Bcl-2 to evolve proteins that look like Bcl-2 but have no Bcl-2-like, anti-apoptotic activity [13].

This suggests that at least many viruses require an anti-apoptotic activity in order to keep the cell alive. This is probably only necessary if a pro-apoptotic activity is generated by the viral infection: that the rate of cell death that naturally occurs in a population of cells would be so high as to endanger replication of the virus seems unlikely. In infection with MVA, a Vaccinia-derived poxvirus, this has been shown directly. MVA has a Bcl-2-like protein, F1L. Human epithelial cells infected with MVA are strongly protected against experimental apoptosis in cell culture. If F1L is deleted genomically in the virus, MVA has not only lost this activity, it in fact induces apoptosis on its own [14]. Taking away the viral anti-apoptotic protein thus unmasks the pro-apoptotic activity of the virus.

Apoptosis is therefore a reaction of the cell to the recognition of the virus. Some of the signalling components required have been described [15], and I will consider this issue further below. It is plausible that apoptosis induced by viruses has evolved as a defence reaction, and a successful virus has been forced to deal with this response.

The point has been made that virus induced apoptosis also is 'used' by the virus for its release (see for instance [11]). This may sound plausible but is, as far as I know, conjecture. Indeed, the mechanism may not be so likely when we think about it more closely. Normally proceeding apoptosis involves the activity of proteases and nucleases, and the dying cell is efficiently taken up by other cells, where the viral material may end up in a degradative pathway. That this mechanism works to permit spreading of the infection has, as far as I know, not been shown.

6. Apoptosis during bacterial infections

Most bacteria do not enter human cells to replicate inside. Some bacteria however are facultative intracellular, and a few are obligate intracellular organisms, that is they can only grow inside mammalian cells. It seems clear that this different life style means that apoptosis will have quite different relevance. An obligate intracellular bacterium is in a similar position as a virus and will be threatened by host cell apoptosis. To an exclusively extracellular bacterium, host cell apoptosis may be of no immediate consequence. I will discuss this issue further below where I will consider some of the pathways known to be active during infection. Individual components of intracellular bacteria and mechanisms by which they can affect the apoptosis apparatus have been reviewed recently [16]. That article in particular illustrates the complexity and uncertainty of this interaction quite beautifully.

7. Pro-apoptotic signals and pro-apoptotic activities

Viral and bacterial, and probably parasite, recognition can induce apoptosis in human cells. This suggests that the receptors that recognize microbes have some connection to the apoptotic pathway.

In innate immunity, that is not considering the recognition by lymphocytes, microbes are recognized by pattern recognition receptors (PRR). PRR recognize microbial molecules that

are shared between large groups of pathogens. LPS is one such molecule (all gram-negative bacteria have LPS and can therefore be recognized); peptidoglycan (almost all bacteria have peptidoglycan) or RNA-molecules with specific structures (many viruses can be recognized that way) are other target structures of immune recognition [17]. Upon binding to their microbial ligands, PRR trigger a set of cell-activating pathways, including the NF-κB-pathway, MAPK-pathways and type I interferon (IFN I)-inducing pathways [18]. However, many of these receptors can also induce apoptosis.

A number of Toll-like receptors (TLR), a group of PRR, have been found to have this principal ability. TLR are transmembrane receptors at the cell surface or in endosomes; their function appears to be to recognize structures of pathogens that reach the cell from the surface (as opposed to microbes replicating inside cells) [18]. In the group of Toll-like receptors, there is a division into two signalling groups: one group uses the signalling adapter MyD88, the other the adapter TRIF (one receptor, TLR4, uses both).

The first report of apoptosis-induction through TLR was published almost twenty years ago: TLR2 caused apoptosis when over-expressed ectopically but also when THP-1 human monocytes were stimulated with a TLR2-agonist [19, 20]. This activity was later not detected in mouse macrophages [21]; it therefore appears that MyD88-dependent TLR have the principal ability to apoptosis and have some connection with the apoptotic apparatus but at the same time are subject to additional regulation through competing pathways.

The induction of apoptosis by TRIF-dependent TLRs has also been reported [21], and this signalling has been characterised more definitively. TRIF uses the same signalling proteins as TNFR1, notably RIPK1, TRADD, cIAPs and caspase-8 [22-24]. The evidence indicates that, like TNFR1, TRIF-dependent receptors have a choice of cell-activating and cell-killing pathways.

In the cytosol, there are PRR that recognize nucleic acids, and they can all induce apoptosis in some circumstances. Two helicases recognise cytosolic RNA (called RIG-I and Mda5) and signal through a mitochondrial adapter, called MAVS. Transfecting synthetic RNA into the

cell initiates a strong signal through this pathway, and this can kill a cell. This approach has even been used to induce apoptosis in tumour cells in a melanoma model in mice [25]. Clearly, this signalling pathway has non-apoptotic functions in innate immunity. There is no getting away from the fact though that it also has principal, substantial pro-apoptotic potential, probably in some cells more than in others.

Several receptors for cytosolic DNA – detecting the DNA of infecting microbes – have been identified. One of them, the cyclic GMP-AMP synthase (cGAS) is now believed to be the most relevant [26]. cGAS signals through a molecule known as stimulator of interferon induced genes (STING), and the cGAS/STING-axis can again induce apoptosis in some circumstances [27], although this is unlikely to be its main function.

Cytosolic peptidoglycan fragments are recognised by yet other PRR, the NOD proteins (NOD1/2). The evidence is relatively weak that these receptors can induce apoptosis. It should be noted however that NOD1 has been found to enhance the activation of caspase-9 when co-transfected [28]. NOD-proteins may therefore also have some pro-apoptotic activity.

I do not believe that apoptosis induction is the main function of any of these PRR. I do however think that this pro-apoptotic activity should be taken seriously.

8. Apoptosis during infection of human cells with microbes: pro- and anti-apoptotic activities at the same time

This seems a very confusing and contradictory issue. Often there appear to be multiple reports concerning one particular pathogen but some authors find that the pathogen induces apoptosis while others suggest that it inhibits apoptosis. Mechanistically and experimentally this is likely the consequence of the different conditions employed. However, whatever the experimental circumstances, it appears that both activities may be vested in one pathogen.

Here are a few examples: *Coxiella burnetii* has been found to inhibit [29] and to induce [30] apoptosis. The same has been shown for *Legionella pneumophila* [31, 32] and *Neisseria*

gonorrhoeae [33, 34]. In *Neisseria* infection, both pro- and anti-apoptotic activities have been detected in the outer membrane protein (porin) PorB (although here porins from two different species, *N. gonorrhoeae* and *N. meningitides* were used [35, 36]). *Chlamydia trachomatis* has well-substantiated anti-apoptotic activity (it protects the infected cell against experimental pro-apoptotic stimuli [37]) but it has also been shown to induce apoptosis [38]. The protozoan parasite *Toxoplasma gondii* has been reported to exhibit both pro- [39] and anti-apoptotic [40] activities in cell culture. The virus MVA induces apoptosis in macrophages and dendritic cells but it inhibits apoptosis in epithelial cells [15, 41].

These examples will suffice to make the point: during an infection with many, and very different pathogens, both pro- and anti-apoptotic activities are detectable.

9. Pro- and anti-apoptotic activities: the cell's perspective

A human cell has, as described above, an arsenal of directly pro- as well as anti-apoptotic proteins (plus many indirect upstream regulators). If a cell wants to die, it can for instance increase the production of the pro-apoptotic BH3-only protein of the Bcl-2-family, Bim. If it wants to inhibit apoptosis, it can choose to up-regulate Bcl-2.

If it is useful to a pathogen to increase or decrease apoptosis, it may through its own components interfere with the host cell's apoptosis apparatus or may interfere with gene expression of the host cell. There are certainly bacterial factors that induce apoptosis in a host cell (good examples are bacterial toxins) but they normally have this as a side effect of interfering with other cellular pathways (see for instance clostridial toxins [42]). I am not aware of any microbe-derived compound that would directly activate the core apoptosis system. There seems to be for instance no known Bax-like protein, or Bim-like protein in a virus or a bacterium. When apoptosis is induced it seems to be through some upstream signalling such as upon PRR-recognition.

There are however quite a number of identified anti-apoptotic proteins. Best investigated are probably the viral Bcl-2-like proteins, which are found in large DNA viruses from different viral

families. There are further examples that are perhaps less well understood. A protein from *Coxiella* has been described that is secreted into the cell and has the capacity to inhibit apoptotic signal transduction at a central step [43]. During infection with *Chlamydia*, an anti-apoptotic activity is generated that, although still molecularly undefined, is almost certainly of bacterial origin.

A further bacterial example is porin B (PorB) of *Neisseria*. This protein has porin function for the bacteria and therefore clearly serves another primary purpose. It has however also been described to have anti-apoptotic activity [36] (as well as pro-apoptotic activity, shown for another species of *Neisseria*, see above [35]). Intriguingly, PorB has been shown to be imported into mitochondria, in a way similar to the voltage-dependent anion channel (VDAC) of mitochondria [44]. VDAC, on the other hand, appears to have anti-apoptotic activity through complex formation with Bak [45]. It is not obvious how these bacteria would benefit from the inhibition of apoptosis since they normally do not live freely in the cytosol (although *Neisseria gonorrhoeae* does transit through human epithelial cells). It may however be an example of how bacteria are able to generate anti-apoptotic activities using porins. Unlike viruses, bacteria do not typically pick up genetic information from eukaryote hosts. They may however, as this example shows, be able to modify their constituents to convey to them an additional function such as apoptosis inhibition. Mitochondria are probably of bacterial origin. There may therefore be relatively little adjustment necessary to make bacterial constituents inhibit or otherwise modify mitochondrial apoptosis.

10. Cui bono? Ways in that apoptosis may help the host

When we look at individual molecular or cellular events of an infection – in apoptosis or otherwise – we will probably always detect steps and processes that will be beneficial to the pathogen and such that will be beneficial to the host. This may even be considered the general scheme of a regular infection: most infections only work if the pathogen can replicate for a while, and if the host in the end is able to clear the infection. If the pathogen cannot

replicate at all it will not manage to cause an infection. If the host is unable to recover, this will in most circumstances not be a stable situation of the infectious agent circulating in the host population. It would therefore be too simple only to look for mechanisms that will benefit infecting viruses: what the virus 'wants' is not complete defeat of the host. What is selected for is replication and transmission in the host population. When we try to understand the role of apoptosis during microbial infection we will have to search for benefits to either side. Will the infectious agent benefit from the induction of apoptosis, or will the host? When apoptosis is inhibited, who will benefit from this situation? While we can easily find situations of both apoptosis-inhibition and apoptosis-induction during infection, I will argue that in general apoptosis is of benefit to the host organism and that apoptosis-induction is an outcome that is part of host defence.

Can we think of ways how apoptosis would benefit the pathogen? The induction of apoptosis in attacking immune cells would be one way but this would be an indirect effect where the pathogen is not in direct replicative contact. Otherwise at least I have difficulty to think of such a way. Release of intracellular pathogens may be a theoretical benefit of apoptosis but I know of no concrete example where such a benefit would have been shown. Tissue damage in the context of an infection is likely to increase inflammation – one can, if pressed hard, think of ways how this would help a specialised pathogen but in general inflammation is not conducive to pathogen replication.

On the other hand, there are more concrete ways how apoptosis can be advantageous to the host. The obvious case is the cell-autonomous defence against obligate intracellular agents, discussed above. The remainder of the possibilities concern the host immune response.

The traditional view, formed over 20 years ago, had been that apoptosis is immunologically silent and does not cause inflammation; sometimes apoptotic cells can even be immunosuppressive [46, 47]. This may be the case when cells simply die by programmed cell death in proliferating tissues, for instance when neutrophils undergo apoptosis at the end of their short life span and are taken up by macrophages. It has however long ago been

recognised that cell death can in fact be immunogenic, depending on the circumstances of the cell death. Numerous individual molecules have been proposed to be involved in such immunogenicity [48]. In this context it is relevant that apoptosis may not be 'pure' but may involve aspects of necroptosis (activity of RIPK-dependent pathways) or at least secondary necrosis (where the plasma membrane is made permeable by DFNA5 [49, 50]). Pathogen-associated as well as host cell components may activate myeloid cells, increasing inflammation and enhancing the innate and indirectly the adaptive immune reaction.

When a host cell dies by apoptosis, during infection or otherwise, its remains are taken up largely by phagocytes. If dendritic cells take up such material, they can present virus-derived material to T cells and thus enhance an anti-viral T cells response [51]. Apoptosis is therefore a way of making microbial antigen available to the adaptive immune cells.

Thus, apoptotic cell death can release pro-inflammatory stimuli, which stimulate components of the innate immune system and can through uptake of material from dying cells by dendritic cells provide material for the presentation to T cells. In a mammalian organism this is likely of general importance.

An intriguing recent development is the demonstration that mitochondrial apoptosis may also, at least in principle, serve to activate a dying cell. Thus, when mitochondria are permeabilized during apoptosis, an IFN I-inducing activity [52, 53] (suggested to be mitochondrial DNA) and an NF-κB-inducing activity [54] are released into the cytosol of the dying cell. The importance of this mechanism at this stage seems limited since the activity could only be observed when caspases were absent or experimentally inhibited; active caspases turned off the cell activation. However, there may be situations where for instance pathogens inhibit caspases, and then this mechanism may be relevant. I think it also conceivable that situations exist where the activity of caspases is too low to turn off this response, and where mitochondrial permeabilisation therefore does activate a cell-autonomous cytokine response, which may trigger inflammation. This is at this stage hypothetical and will require experimental confirmation. However, I believe that the sum of

the evidence supports the view that a principal feature of apoptosis is to support initiation of an anti-microbial immune response.

11. Conclusion: the role of apoptosis during microbial infection

In summarizing these considerations, the following is my personal view, and it is a biased view:

Pathogens often carry anti-apoptotic activities but not typically (as far as we know) directly pro-apoptotic molecules. Although anti-apoptotic pathways are also triggered by microbe derived material (most activation associated pathways in human cells carry some anti-apoptotic potential), the dedicated pathogen recognition pathways, in particular PRR-dependent pathways, have pro-apoptotic potential. This suggests that apoptosis is, beyond its obvious use to get rid of obligate intracellular pathogens, generally a defence reaction that is of benefit to the host.

Is there an overarching benefit to the host? In my personal perception the immunostimulatory potential is the most important aspect. One aspect is the delivery of antigen for presentation to the adaptive immune system. At least as important may be the pro-inflammatory potential of apoptotic cell death. This mechanism may involve microbial molecules that are made available to the immune system. It may also be the generation of pro-inflammatory stimuli that may cell-autonomously act to activate the cell, or secreted (or otherwise released) molecules that alert professional immune cells to the infection.

We still don't know what the original function of apoptosis was, when it first appeared in evolution. We do have a clearer idea of some of the functions it has in complex animals. Immune modulation and in particular inflammation and initiation of an immune response would seem a good application of an evolutionarily old concept to more recent principles of organismic function.

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