Restricted Theiler’s murine encephalomyelitis virus infection in murine macrophages induces apoptosis

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Increasing evidence suggests that macrophages (Møs) are necessary for persistence of Theiler’s murine encephalomyelitis virus (TMEV) in the mouse central nervous system. Analysis of BeAn virus infection in the Mø cell lines P388D1, J774A.1 and PU5-1.8, which are intermediate in their state of differentiation and resemble multifunctional resident Møs, revealed restricted TMEV growth. As a result of the restricted infection, these Mø cell lines were induced to undergo apoptosis as demonstrated by cellular morphology, DNA fragmentation, caspase protease activity, and in individual cells, by terminal deoxytransferase dUTP nick-end labelling (TUNEL).

The Theiler’s murine encephalomyelitis viruses (TMEVs) can be divided into two groups based on their neurovirulence characteristics after intracerebral inoculation of mice. Highly virulent strains, such as GDVII virus, cause acute fatal encephalitis in mice. The less virulent strains, such as BeAn and DA viruses, are characterized by at least a 102-fold increase in mean 50% lethal dose compared with the virulent group and by their ability to persist in the central nervous system (CNS) of mice. The literature indicates that TMEV persistence leads to damage of myelin, which is largely immunologically mediated by major histocompatibility (MHC) class II-restricted Th1 lymphocytes directed at virus epitopes (Miller et al., 1987, 1989; Gerety et al., 1994a, b; Clatch et al., 1986).

Macrophages (Møs) are critical cells in host defence, since they participate not only in innate immunity, but also in antigen-specific immune responses. Virus-infected Møs secrete cytokines which augment the local inflammatory response and also process and present virus antigen(s) to MHC class I- and II-restricted T cells. Møs are long-lived cells which are also principal targets for persistent infection by a wide variety of pathogens, e.g. bacteria, viruses and parasites (Kaufman, 1993; Wu et al., 1990). During TMEV persistence, the major virus antigen burden is found in CNS Møs (Lipton et al., 1995; Pena Rossi et al., 1997); however, only a small percentage of the Møs infiltrating demyelinating lesions contain virus antigen (Lipton et al., 1995). Depending on the state of differentiation, we have also found that murine monocyte/macrophage cell lines may be resistant (M1 cells) or semi-permissive (RAW264.7 and P388D1) to TMEV infection (Jelachich et al., 1995). Recently, we found that TMEV induces apoptosis in restrictive BSC-1 cells, in which TMEV replication is similar to that in Møs, but not in permissive BHK-21 cells (Jelachich & Lipton, 1996). Therefore, we examined whether BeAn virus infection would induce apoptosis in murine Mø cell lines.

Three Mø cell lines, J774A.1, P388D1 and PU5-1.8, purchased from the ATCC and which mediate antibody-dependent cell-mediated cytotoxicity and/or spontaneous tumour lysis, are phagocytic and F4/80+ Mac-1+, FcR+, C3R+ and MHC class II+, were examined for their response to BeAn virus infection. Based on their morphology, plastic adherence and regulation of MHC class II expression, these cell lines represent Møs that are intermediate in their state of differentiation, resembling multifunctional resident Møs (Mauel & Defendi, 1971; Taniyama & Holden, 1980; van Furth et al., 1985). BeAn virus infection in these cell lines resulted in a low percentage of cell survival 24 h after infection, as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Fig. 1A). The MTT assay determines mitochondrial activity and is an indicator of cell viability (Jelachich & Lipton, 1996). Analysis of the morphology of J774A.1, P388D1 and PU5-1.8 cells after infection indicated that cell death occurred by apoptosis. The nuclei became condensed and fragmented and the cells contracted as shown by propidium iodide staining in Fig. 2. In addition, distinct nucleosomal-sized DNA bands were visible by gel electrophoresis in each of the cell lines following infection (Fig. 1B). Flow cytometry using TdT and BrdUrd to label 3’ nicked DNA ends and propidium iodide staining to determine total cellular DNA content (Li & Darzynkiewicz, 1995) also revealed DNA nicking and degradation characteristic of apoptosis in each of the infected Mø cell lines 24 h after infection (Fig. 1C). The percentage of cells with intact DNA and those with nicked...
and degraded DNA varied somewhat among the cell lines; 82% to 98% of the infected MΦs had nicked and degraded DNA, while only 2% to 18% contained intact DNA. It is not known whether the DNA in cells with only strand breaks (upper right quadrants) is repaired or undergoes degradation, i.e. whether the cells are committed to apoptosis. The relative sensitivity to apoptosis as determined by flow cytometry paralleled that observed in the MTT assay of cell survival (Fig. 1 A).

The temporal profile of DNA nicking and degradation was examined by flow cytometry at 4, 8, 12 and 24 h after infection for the PU5-1.8 and J774A.1 cell lines. Although these cell lines differed somewhat in their sensitivity to virus-induced apoptosis (Fig. 1 A), both showed the same temporal profile with DNA strand nicks first detected at 12 h after infection (not shown). Further support for the induction of apoptosis by BeAn virus was the detection of caspase activity in supernatants from infected MΦ cell lines. Although the earliest increase in caspase activity that could be measured varied with the cell line (between 4 and 8 h; not shown), caspase activity peaked 12 h after infection when DNA strand nicks first appeared (Fig. 1 D).

Previously, we showed that BeAn virus infection in RAW 264.7 and P388D1 cells is highly restricted (Jelachich et al., 1995). Analysis of BeAn virus RNA replication by incorporation of [3H]uridine into nascent virus RNA in the presence of 5 µg/ml actinomycin D in the three cell lines indicated a kinetics of virus RNA replication similar to that observed in permissive BHK-21 cells; however, the amount of virus RNA synthesized was 120- to 400-fold less (Fig. 3 A). These data indicate that although TMEV initiates virus RNA replication at the same time as in productively infected BHK-21 cells, virus RNA replication is restricted in the murine MΦ cell lines. Measurement of infectious virus by standard plaque assay showed a kinetics similar to that in BHK-21 cells, with peak virus titres reached at 8 to 12 h (Fig. 3 B). However, unlike permissive BHK-21 cells, virus titres declined after 12 h in the MΦ cell lines. These titres were also at least 300-fold less than those in BHK-21 cells. Although little infectious virus was produced, as shown in Fig. 3 (B), > 95% of the cells infected with BeAn virus expressed substantial amounts of virus antigen, which was typically compacted into an oval profile in the cytoplasm (Fig. 2). Thus, BeAn virus infection is restricted at least in viral RNA replication and assembly into infectious particles.

MΦs are known to induce apoptosis in other cells by production of TNF-α and nitric oxide (Voelkel-Johnson et al., 1995; Grell et al., 1994; Wu et al., 1995; Aliprantis et al., 1997).
TMEV-induced apoptosis in murine MΦs

Fig. 2. Double immunofluorescent staining of J774A.1 (A, C) and PU5-1.8 (B, D), either mock-infected (A, B) or infected with BeAn virus at an m.o.i. of 10 (B, D), showed co-localization of virus antigen to cells with condensed and fragmented nuclei (arrows show most prominent examples of cell shrinkage antigen condensation). The cells were fixed in 1% paraformaldehyde, permeabilized and stained with biotinylated anti-BeAn rabbit IgG and strepavidin AMCA for virus antigen and propidium iodide for DNA as described (Jelachich et al., 1995). Magnification ×630.

and also to undergo apoptosis under conditions of infection (Monack et al., 1996), perturbation of growth signals (Mangan et al., 1991; Mangan & Wahl, 1991) or in the presence or absence of inflammatory cytokines (Munn et al., 1995). We find here that murine MΦ cell lines resembling resident MΦs are susceptible to apoptotic cell death following infection with TMEV.

Apoptosis of MΦs and T cells has been reported to occur in the CNS of Lewis rats with experimental allergic encephalomyelitis (EAE) (Pender et al., 1992; Nguyen et al., 1994). EAE in rats resolves after an initial inflammatory response and the animals are tolerant to further induction of EAE. On this basis, it was hypothesized that myelin basic protein-specific T cells are eliminated so that the host cannot initiate another antigen-specific response. TMEV-induced demyelination is a chronic process in which virus persists for many months; thus, tolerance to the initiating antigen is never achieved. Apoptosis in this case may simply be responsible for the chronicity of the inflammatory response. Tsunoda et al. (1997) reported finding apoptosis of MΦs and oligodendrocytes in demyelinating lesions of DA virus-infected mice; however, very few apoptotic MΦs were observed and they did not specifically demonstrate virus antigen in these apoptotic cells. Therefore, there is no evidence as yet of infected cells in the CNS of a TMEV-infected mouse undergoing apoptosis.

Accumulating evidence points to a central role of MΦ infection in TMEV persistence in the mouse CNS (Clatch et al., 1990; Levy et al., 1992; Jelachich et al., 1995; Lipton et al., 1995; Pena Rossi et al., 1997). Pena Rossi et al. (1997) recently provided further support for this notion by their demonstration that depletion of MΦs by mannosylated liposomes almost completely abrogates persistence of TMEV. The restricted state of BeAn virus replication in our murine MΦ cell lines resembles that reported for CNS MΦs in mice (Clatch et al., 1990; Cash et al., 1985). In addition, other investigators have also found that TMEV infection in murine MΦ cell lines is restricted (Obuchi et al., 1997; Shaw-Jackson & Michiels, 1997; Takata et al., 1998). Based on our in vitro studies, TMEV growth in MΦs may be restricted at a number of steps in the replicative cycle; however, the exact details and mechanism(s) involved are unclear.

The kinetics of BeAn virus replication in murine MΦs (peak by 8 to 12 h, declining thereafter) is similar to the kinetics in permissive BHK-21 cells and is indicative of an acute infection.
Although Mφs are long-lived cells, there is no evidence as yet of persistent TMEV infection of Mφs in vitro (Levy et al., 1992). In fact, the data presented here suggest that infection of Mφs rapidly leads to cell death by apoptosis. Since it is unlikely that TMEV replicates for days or weeks in individual Mφs, spread of virus from Mφ to Mφ may be necessary to perpetuate the infection in the mouse CNS. In this setting, apoptosis of infected Mφs probably represents a cellular defence mechanism through the premature death of infected cells. The susceptibility to apoptosis in two of our Mφ lines, e.g. P388D1 and PUS5-1.8 cells, appeared to limit BeAn virus replication. Alternatively, such an event might also foster virus spread and persistence. Phagocytosis of an infected apoptotic Mφ by an uninfected neighbour could initiate infection in the phagocytic cell. In this way, TMEV could spread from cell to cell to perpetuate the infection without extracellular exposure of virus to the immune system.

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References


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