TLR3 Can Directly Trigger Apoptosis in Human Cancer Cells

Bruno Salaun, Isabelle Coste, Marie-Clotilde Rissoan, Serge J. Lebecque and Toufic Renno

*J. Immunol.* 2006;176:4894-4901
http://www.jimmunol.org/cgi/content/full/176/8/4894

References

This article cites 44 articles, 20 of which can be accessed free at: http://www.jimmunol.org/cgi/content/full/176/8/4894#BIBL

15 online articles that cite this article can be accessed at: http://www.jimmunol.org/cgi/content/full/176/8/4894#otherarticles

Subscriptions

Information about subscribing to *The Journal of Immunology* is online at http://www.jimmunol.org/subscriptions/

Permissions

Submit copyright permission requests at http://www.aai.org/ji/copyright.html

Email Alerts

Receive free email alerts when new articles cite this article. Sign up at http://www.jimmunol.org/subscriptions/etoc.shtml
TLR3 Can Directly Trigger Apoptosis in Human Cancer Cells

Bruno Salaun, Isabelle Coste, Marie-Clotilde Rissoan, Serge J. Lebecque, and Toufic Renno

TLRs function as molecular sensors to detect pathogen-derived products and trigger protective responses ranging from secretion of cytokines that increase the resistance of infected cells and chemokines that recruit immune cells to cell death that limits microbe spreading. Viral dsRNA participate in virus-infected cell apoptosis, but the signaling pathway involved remains unclear. In this study we show that synthetic dsRNA induces apoptosis of human breast cancer cells in a TLR3-dependent manner, which involves the molecular adaptor Toll/IL-1R domain-containing adapter inducing IFN-β and type I IFN autocrine signaling, but occurs independently of the dsRNA-activated kinase. Moreover, detailed molecular analysis of dsRNA-induced cell death established the proapoptotic role of IL-1R-associated kinase-4 and NF-κB downstream of TLR3 as well as the activation of the extrinsic caspases. The direct proapoptotic activity of endogenous human TLR3 expressed by cancerous cells reveals a novel aspect of the multiple-faced TLR biology, which may open new clinical prospects for using TLR3 agonists as cytotoxic agents in selected cancers. The Journal of Immunology, 2006, 176: 4894–4901.

The recently identified TLR family consists of a germline-encoded set of molecules thought to be critically involved in the detection of pathogens and the triggering of an immune response against microbial infections (1). Ligation of TLRs by their respective ligands triggers well-characterized signaling cascades that result in activation of downstream effectors, such as NF-κB, p38, JNK, and IFN regulatory factors (IRFs) (2); resistance against pathogens (3); and, occasionally, cell death (4), which is another way of protecting the host against microbe spreading (5). Such proapoptotic properties have indeed been demonstrated for TLR2 and TLR4, which can induce apoptosis in macrophages through signaling via the molecular adaptor MyD88 and are involved in the adjuvant role of dsRNA in tumor vaccination, most notably through the promotion of Ag cross-presentation by dendritic cells and the induction of enhanced primary and memory CD8$^+$ T cell responses (16, 17). Recent studies in mouse models have highlighted the adjuvant role of dsRNA in tumor vaccination, most notably through the promotion of Ag cross-presentation by dendritic cells and the induction of enhanced primary and memory CD8$^+$ T cell responses (16, 17). Recent studies in mouse models have highlighted the adjuvant role of dsRNA in tumor vaccination, most notably through the promotion of Ag cross-presentation by dendritic cells and the induction of enhanced primary and memory CD8$^+$ T cell responses (16, 17).

3 Address correspondence and reprint requests to Dr. Serge J. Lebecque at the current address: Hospices Civils de Lyon, Centre Hospitalier Lyon Sud Service de Pneumologie (Bat. 5F), 165 chemin du Grand Revoyet, 69495 Pierre-Bénite, Cedex, France. E-mail address: serge.lebecque@chu-lyon.fr

Received for publication October 12, 2005. Accepted for publication January 12, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Materials and Methods

Cell lines and reagents

Human breast tumor cell lines (Cama-1, SW527, BT-483, and MCF-7) were obtained from the American Type Culture Collection and cultured in DMEM/Ham’s F-12 medium containing 4.5 g/ml glucose (Invitrogen Life Technologies) supplemented with 2 mM L-glutamine (Invitrogen Life Technologies), 10% FCS (Invitrogen Life Technologies), 2.5 mg/ml sodium bicarbonate (Invitrogen Life Technologies), amino acids (Invitrogen Life Technologies), and 1 mM sodium pyruvate (Sigma-Aldrich; referred to as complete medium). Polyinosinic-polycytidylic acid (poly(I:C)) was obtained from InvivoGen. Peptidoglycan and LPS were purchased from Sigma-Aldrich. Type I IFN-blocking mAb was purchased from PBL Biochemical Laboratories, and TNF-α-neutralizing mAb was obtained from Genzyme. Abs to Stat-1, phosphorylated Stat-1 (Tyr701), and PKR were purchased from Cell Signaling Technology. Abs to human IFN-β were obtained from R&D Systems, and Abs to NF-κB p65 subunit, TNFR-associated factor 6 (TRAF6), and β-tubulin were purchased from Santa Cruz Biotechnology. The general caspase inhibitor z-Vad-Ala-Asp(Ome)-fluoromethyl ketone (z-VAD-fmk) was purchased from R&D Systems, and cycloheximide (CHX) was obtained from Sigma-Aldrich.

Copyright © 2006 by The American Association of Immunologists, Inc. 0022-1767/06/$02.00
Human primary breast tumor samples were obtained from the Centre Léon Bérard in agreement with the hospital’s bioethical protocols. Single-cell suspensions were obtained after digestion with collagenase A (Sigma-Aldrich) and enrichment in human epithelial Ag (HEA)-positive cells using HEA microbeads (Miltenyi Biotec) according to the manufacturer’s instructions. The final single-cell suspension contained >80% HEA-positive cells and <2% CD45<sup>+</sup> hemopoietic contaminants.

Apoptosis and proliferation analysis

Cell recovery after treatment with TLR ligands was measured by crystal violet staining (Sigma-Aldrich). Cells were plated at 10<sup>4</sup> cells/well in 96-well plates, and after 72-h culture with or without TLR ligand, cells were washed with PBS, fixed in 6% formaldehyde (Sigma-Aldrich) for 20 min, washed twice, and stained with 0.1% crystal violet for 10 min. After washes and incubation in 1% SDS for 1 h, absorbance was read at 605 nm on a Vmax plate reader (Molecular Devices). Annexin V staining was performed with an Annexin<sup>FITC</sup> apoptosis detection kit (BD Pharmingen) according to the manufacturer’s instructions. Subdiploid cells were detected by staining with 3 μg/ml propidium iodide (PI; Molecular Probes) after overnight permeabilization in 70% ethanol. Fluorescence was analyzed by flow cytometry on a FACSCalibur (BD Biosciences) equipped with a doublet-discrimination module using CellQuest Pro software (BD Biosciences). Cama-1 cell proliferation was analyzed with the anti-BrdU FITC-conjugated Ab set (BD Pharmingen) after a 1-h pulse with 10 μg/ml BrdU (Sigma-Aldrich) according to the manufacturer’s instructions.

ELISA

Production of IL-6 by Cama-1 was assessed in culture supernatants with the DuoSet ELISA kit (R&D Systems) according to the manufacturer’s instructions.

Biochemistry

Cama-1 cells were lysed in 1% Nonidet P-40-containing buffer, and 20 μg of total proteins were loaded per lane on SDS-polyacrylamide gels (Invitrogen Life Technologies). Western blots (WB) were performed with standard techniques using the Abs described above.

Small interfering RNA (siRNA) experiments

Cama-1 cells were plated in six-well plates at 3 × 10<sup>5</sup> cells/well. After overnight adherence, siRNA transfections were performed for 5 h in OptiMEM medium (Invitrogen Life Technologies) containing 3 μg/ml Lipofectamine 2000 (InvivoGen) and 100 nM siRNA. Cells were then washed and cultured for 72 h in complete medium before treatment with poly(I:C) and apoptosis analysis. The siRNA duplexes specific for TLR3, PKR, and p65 were purchased from Dharmacon as SMART-Pools. TRIF and control scrambled siRNA were purchased from the same supplier as single oligo-duplexes (5'-GCUCUUGUAUCUGAAGCAC-3' and 5'-ACUAGUCGACGACGCC-3', respectively). TLR3 and TRIF expressions were assessed by PCR (35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C) with Taq PCR ReadyMix (Sigma-Aldrich) using the following primers: 5'-AACGATTCCTTTGCTTGGCTTC-3' (forward)/5'-GCTTAGACCTTGCTGGGCTTC-3' (reverse).

FIGURE 1. Synthetic dsRNA induces TLR3- and TRIF-dependent apoptosis of human breast tumor cells. a, Breast tumor cell recovery after culture with poly(I:C) is expressed as a percentage, with cultures in medium alone considered 100%. The data shown were obtained from three independent experiments conducted in triplicate. The star indicates a statistical difference from respective controls (p < 0.05). b, Cama-1 cells were cultured for 24 h without (gray) or with (white) poly(I:C), and apoptosis was measured by annexin V staining. c, Cama-1 cells were cultured without (△) or with increasing doses of poly(I:C) (□, 0.5 ng/ml; ○, 5 ng/ml; ■, 50 ng/ml; ■, 500 ng/ml; ●, 5 μg/ml), and the percentage of annexin V-positive apoptotic cells was measured at the indicated time points. Data shown are representative of three independent experiments with similar results. d, Cama-1 cells were cultured without (PBS) or with poly(I:C), and DNA content was measured by PI staining. The percentage indicates the proportion of subdiploid cells in cultures. e, Cama-1 cells were cultured for 24 or 48 h without (□) or with (■) poly(A:U). Annexin V-positive apoptotic cells are expressed as a percentage of the total cells in culture. Data shown were obtained from three independent experiments. f, Freshly recovered breast tumor cells were cultured in medium without (PBS) or with poly(I:C), and cell DNA content was measured by PI staining. Percentages indicate the proportion of cells with low DNA content (subdiploid cells), i.e., apoptotic cells.
TCCGAATGGTCAAG-3′ (reverse) for TLR3 and 5′-ACTTCTCTAG CGCCTCTGGAC-3′ (forward)/5′-ATCTTCTACAGAAAGTTTGA-3′ (reverse) for TRIF. The expressions of PKR and p65 were assessed by WB as described above.

Statistics
Statistical significance was assessed with two-tailed Student’s t test, and results are given as the mean ± SD.

Results
TLR3 agonists can directly trigger apoptosis in breast cancer cell lines
To investigate the role of TLR3 agonists on tumor cells, human breast adenocarcinoma cell lines were cultured with 50 μg/ml of the dsRNA analog poly(I:C) for 72 h. Three of four cell lines tested (Cama-1, BT-483, and SW527, but not MCF-7) showed a significant decrease in cell recovery, as measured by crystal violet staining, with Cama-1 consistently exhibiting the most dramatic drop (Fig. 1a). Nevertheless, the polyIC-induced decrease in cell recovery of BT483 and SW527, although weaker than that in CAMA-1 cells, was both significant (35 and 25%, respectively, compared with <3% in controls) and highly reproducible (at least three times). The decrease in recovery of Cama-1 cells was due to apoptosis, as illustrated by annexin V staining (Fig. 1b). Poly(I:C) triggered significant dose-dependent apoptosis in the Cama-1 cell line, starting at 9 h and reaching a level of 80% apoptotic cells after 30 h of treatment (Fig. 1c). Cell recovery decrease was associated with an increase in subdiploid cells, as illustrated by PI staining (Fig. 1d). When added at 50 μg/ml to Cama-1 cell culture, the dsRNA analog polyadenylic-polyuridylic acid (poly(A:U)) triggered similar cell death, although with slower kinetics (Fig. 1e).

Poly(I:C)-induced tumor cells apoptosis is mediated by TLR3 and TRIF, but occurs independently of PKR
PKR can be triggered by synthetic transfected dsRNA (21), whereas TLR3 can be triggered by exogenous poly(I:C) (22). To determine whether PKR or TLR3 was involved in dsRNA-induced Cama-1 cells apoptosis, the expression of each protein was efficiently suppressed through transfection of specific siRNAs (Fig. 2a). Interestingly, although TLR3 mRNA was not readily detected in the steady state in either Cama-1 cells or the other cell lines studied, and the level of TLR3 mRNA, as evaluated by PCR, was not directly linked to the apoptotic response to poly(I:C) in the four lines analyzed (Fig. 2a and data not shown), poly(I:C) treatment induced strong TLR3 mRNA up-regulation in Cama-1 cells (Fig. 2d).
2a, left panel). Suppression of TLR3 with specific siRNA virtually abrogated poly(I:C)-induced apoptosis, whereas cell death occurred normally in the almost complete absence of PKR (Fig. 2b).

The serine/threonine protein kinase inhibitor 2-amino purine had no effect on poly(I:C)-induced apoptosis (data not shown), confirming the lack of PKR involvement. Although the involvement of MyD88 in TLR3 signaling remains controversial, TRIF is the critical adaptor protein for TLR3 from which signaling diverges. On the one hand, the recruitment of TRAF6 and receptor-interacting protein 1 by TRIF leads to the activation of NF-κB, JNK, and p38. In contrast, recruitment and activation of TRAF family member-associated NF-κB activator binding kinase drive the nuclear translocation of IRF-3 and the production of type I IFN (1, 23, 24). Accordingly, suppression of TRIF, but not MyD88, with specific siRNA significantly reduced poly(I:C)-induced apoptosis of Cama-1 cells (Fig. 2b). Double-stranded RNA not only induced apoptosis, but also blocked the proliferation of Cama-1 cells, as measured by BrdU incorporation (Fig. 2c). The siRNA experiments showed that, like apoptosis, the cytostatic effect of poly(I:C) was mediated by TLR3, but was independent of PKR. Of note, inhibition of TRIF or MyD88 expression by itself decreased BrdU incorporation (Fig. 2c), suggesting a role for these molecules in Cama-1 cell proliferation. Taken together, these data demonstrate that synthetic dsRNA both induces the apoptosis and blocks the proliferation of breast cancer Cama-1 cells in a TLR3- and TRIF-dependent manner, which involves neither PKR nor MyD88.

**TLR3-mediated tumor cells apoptosis requires type I IFN signaling**

Because TRIF adapter is known to mediate the type I IFN response of TLR3 (23), the role of type I IFN in TLR3- and TRIF-mediated apoptosis was evaluated. IFN-β production was strongly induced upon poly(I:C) treatment, and Stat1 phosphorylation was observed, indicative of type I IFN signaling (Fig. 3a). Of note, the very sensitive detection of Stat1 phosphorylation was maximum after 6 h of poly(I:C) treatment, when IFN-β production was still hardly detectable by WB. Neutralization of type I IFN with specific mAb significantly reduced poly(I:C)-induced apoptosis (Fig. 3b), demonstrating that type I IFNs were necessary for TLR3-mediated cell death. However, treatment of Cama-1 cells with a mixture of IFN-α and IFN-β did not induce apoptosis (Fig. 3b), whereas it sensitized other breast cancer cells to apoptosis, thereby demonstrating its biological activity (B. Salaun and S. J. Lebecque, manuscript in preparation). These results establish that type I IFN signaling is required for TLR3-triggered cytotoxicity, although it is insufficient to induce cell death by itself. Therefore, type I IFN- and additional TLR3-triggered signaling pathways appear to cooperate to trigger Cama-1 cells apoptosis.

**TLR3-induced cell death is mediated by IL-1R-associated kinase 4 (IRAK-4) independently of TRAF6**

Besides type I IFN production, TLR3 has also been shown to trigger TRIF-mediated NF-κB activation (12). IRAKs are central to TLR signaling and are known to induce IκB degradation through TRAF6 recruitment and subsequent activation of the IκB kinase complex (2). However, the roles of IRAK-4 and TRAF6 in TLR3 signaling remain unclear. The siRNAs specific for each molecule efficiently suppressed the expression of the respective protein in Cama-1 cells (Fig. 4a). Double-stranded RNA-induced IL-6 secretion, which is mediated by TLR3 (Fig. 4b), was significantly reduced in the absence of either IRAK-4 or TRAF6 expression. Unexpectedly, IRAK-4, but not TRAF6, suppression prevented poly(I:C)-triggered and TLR3-mediated apoptosis (Fig. 4c). Incidentally, the very low secretion of IL-6 by Cama-1 cells not exposed to poly(I:C) indicated that siRNA did not significantly activate TLR3. Taken together, these results indicate that both IRAK-4 and TRAF6 participate in the endogenous TLR3 signaling in Cama-1 cells and reveal an unsuspected pathway, downstream of TLR3, that involves IRAK-4, but not TRAF6, and leads to cell death.

**TLR3-mediated cytotoxicity occurs independently of TNF-α, but requires NF-κB and protein synthesis**

An autocrine effect of TNF-α has previously been implicated in the apoptotic activity of TLR4 ligand in human alveolar macrophages (25). This cytokine plays no role in TLR3-mediated apoptosis, because neutralizing anti-TNF-α Ab, which protects Cama-1 cells from TNF-α-induced apoptosis, has no effect on poly(I:C)-triggered cell death (Fig. 5a). The general transcriptional inhibitor CHX is known to sensitize cells to TNF-α-induced apoptosis by blocking the NF-κB-controlled survival program (26). As expected, pretreatment with CHX significantly sensitized Cama-1 cells to TNF-α-induced cytotoxicity (Fig. 5a). In contrast, it partially protected the cells against poly(I:C)-triggered apoptosis, confirming that different mechanisms were triggered by these two pro-apoptotic stimuli. Indeed, inhibition of NF-κB p65 expression by specific siRNA (Fig. 5b) protected Cama-1 cells against poly(I;
Collectively, these results demonstrate that TNF-α secretion is not responsible for poly(I:C)-induced apoptosis and establish a proapoptotic role of NF-κB in TLR3-mediated apoptosis that contrasts with its antiapoptotic function upon TNF treatment.

Extrinsic caspases mediate TLR3-induced apoptosis

The role of caspases in poly(I:C)-induced cell death was analyzed. The broad caspase inhibitor, z-VAD-fmk, which inhibited TNF-α-induced cell death, also greatly reduced poly(I:C)-triggered apoptosis, suggesting a major role for caspases in TLR3-mediated cytotoxicity (Fig. 6a). Poly(ADP-ribose) polymerase (PARP) cleavage, a hallmark of caspase-dependent apoptosis, occurred in Cama-1 cells upon poly(I:C) treatment (Fig. 6b, top panel), confirming the involvement of caspases in TLR3-mediated apoptosis. Caspase 3 was indeed activated upon poly(I:C) treatment, as demonstrated by WB analysis (Fig. 6b, middle panel). Interestingly, caspase 8 also was activated by poly(I:C) (Fig. 6b, lower panel), reminiscent of the apoptosis triggered by TRIF overexpression (10), and the caspase 8-specific inhibitor z-IETD prevented the apoptosis (data not shown).

The low levels of activated caspases 3 and 8 still present after z-VAD-fmk pretreatment and poly(I:C) stimulation may be responsible for the residual apoptosis observed by annexin staining, although the involvement of a caspase-independent apoptotic pathway remains a possibility. Caspase 9 activation could not be detected (data not shown), although poly(I:C) triggered a sharp
decrease in mitochondrial membrane potential, as measured by 3,3′-dihexyloxacarbocyanine iodide (3) staining (Fig. 6c), and a clear up-regulation of the proapoptotic Bax protein (Fig. 6d). Taken together, these results demonstrate the dominant role of the extrinsic apoptotic pathway (shared with death receptors such as TNFR, Fas, and TRAIL) in poly(I:C)-triggered apoptosis, although some participation of the intrinsic pathway could not be completely excluded (27).

**Discussion**

Although involvement of TLR3 in apoptosis has recently been suggested (9, 10, 28), direct demonstration of the participation of this receptor in cancer cell apoptosis is lacking. The present work demonstrates the role of TLR3 in triggering breast cancer cell apoptosis via the adaptor TRIF, independently of PKR and MyD88.

In addition to TLR3 and PKR, the RNA helicase retinoic acid-inducible gene 1 (RIG-1) was recently described to initiate a cellular response to dsRNA (29). However, TLR3 and RIG-1 are reported to trigger nonoverlapping signaling pathways. Therefore, given the almost complete protection provided by either TLR3 or TRIF siRNAs in Cama-1 cells, it is unlikely that RIG-1 plays an important role in dsRNA-induced apoptosis. Molecular events involved in cell death induced by TLR3 agonists include the production of type I IFN, which is required, but not sufficient, for apoptosis. NF-κB p65 and extrinsic caspases are activated by TLR3 engagement and are also necessary for TLR3-mediated apoptosis. Regarding the signaling pathway, we demonstrate in this study that IRAK-4 and TRAF6 are involved in TLR3-triggered IL-6 production by Cama-1 cells. Although transfection-based studies have excluded IRAK-4 from TLR3-triggered signaling cascade (30), our data are in agreement with reports demonstrating that the lack of IRAK-4 expression deeply affects the response to dsRNA in both mice (31) and humans (32). Poly(I:C)-induced cell death also reveals a pathway downstream of TLR3 that signals through IRAK-4 even in the absence of TRAF6. Similar to our findings, a branching point downstream of IRAK kinases has been described for TLR4, where proapoptotic and NF-κB signalizations were shown to diverge after IRAK-1 activation (33).

However, several steps along the proapoptotic signaling pathway induced by TLR3 remain to be clarified. Indeed, it is unclear whether the early recognition of dsRNA is mediated by the low level of TLR3 expressed on resting cells or by another receptor. Elucidating the exact contribution of IFNRI signaling (known to activate the extrinsic caspases (34)) and analyzing the putative roles of proteins such as TBK1, IRF-3, and RIP1, which all participate in TLR3 signaling (35) will also require additional investigations. Type I IFNs involvement is reminiscent of the toxicity of the combination of dsRNA and type I IFNs for many cell types (36) and of the essential role these cytokines play in PKR-independent, virus-induced, apoptotic cell death (37). Regarding the mechanisms of action, the partial inhibition of dsRNA-induced
apoIptosis by the protein synthesis general inhibitor CHX shows that type I IFNs do not participate in TLR3-triggered cell death simply by down-regulating protein synthesis through PKR-in-
duced phosphorylation of eukaryotic initiation factor2α. Alternatively, type I IFNs can facilitate apoptosis in various cell types by up-regulating the expression of proteins directly involved in cell
death, including caspases (38), TRAIL, and p53 (39). Furthermore, IFN-α induces the expression of multiple genes that increase and accelerate the response to dsRNA, including PKR, 2′,5′-oligoad-
enuate synthetase, IRF-3, and TLR3 (15). Lastly, in contrasts with its survival role after TLR2 (40) and TLR4 (7) triggering, NF-κB appears to be required for TLR3-induced apoptosis. It remains to be established whether the p65 subunit of NF-
κB might have a direct proapoptotic effect on tumor cells. Indeed, against cancer cells, the above data suggest that TLR3 agonists
stimulation may account for the variation in sensitivity observed in vitro.

Both poly(I:C) and poly(A:U) have been used with moderate success as adjuvant therapy in clinical trials for different types of cancer, including adenocarcinomas of the breast (42). Although the initial goal had been to trigger an innate immune response against cancer cells, the above data suggest that TLR3 agonists might have a direct proapoptotic effect on tumor cells. Indeed, retrospective immunostaining of breast tumor biopsies has shown that only patients with TLR3 expression had a prolonged survival after receiving poly(A:U) vs placebo (43, 44). Those results support a direct effect of TLR3 agonist on cancer cells that is compatible with our in vitro data and that, in contrast to other reports of TLR-triggered apoptosis, does not require simultaneous inhibition of transcription, translation, or proteasomal degradation (9, 28, 33). Importantly, although we could not obtain primary normal breast cells for in vitro study, the lack of breast side effects in patients receiving TLR3 agonist after surgical removal of their tumor (16) is encouraging considering the possible toxicity of such treatment on nontransformed breast epithelial cells.

To conclude, the present data open a new range of therapeutic applications for TLR3 agonists as cytotoxic agents in selected cancers and raise the exciting concept of multifunctional adjuvants that are able to both directly kill the tumor and enhance the host’s immune response against it.

Acknowledgments
We gratefully thank Jean-Yves Blay and Christine Caux-Ménétier for pro-
viding fresh tumor samples, Jean-Jacques Pin for invaluable technical help, and Sem Sacland and Blandine de Saint-Vis for critical reading of the manuscript.

Disclosures
The authors have no financial conflict of interest.

References
stranded RNA-dependent protein kinase (PKR) involves the α subunit of eukary-
β-cell apoptosis and up-regulates caspase-12 and tumor necrosis factor receptor-
21. D Orthodox antagonist on
22. Miechew, O., and J. Tschopp. 2003. Induction of TNF receptor I-mediated ap-

4900 TLR3 CAN TRIGGER APOPTOSIS


