A Role for the NF-κB Pathway in Cell Protection from Complement-Dependent Cytotoxicity

Dana Gancz, Michal Lusthaus and Zvi Fishelson

*J Immunol* 2012; 189:860-866; Prepublished online 8 June 2012;
doi: 10.4049/jimmunol.1103451
http://www.jimmunol.org/content/189/2/860

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/06/08/jimmunol.1103451.DC1

References
This article cites 46 articles, 13 of which you can access for free at:
http://www.jimmunol.org/content/189/2/860.full#ref-list-1

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

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2012 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
A Role for the NF-κB Pathway in Cell Protection from Complement-Dependent Cytotoxicity

Dana Gancz, Michal Lusthaus, and Zvi Fishelson

Nucleated cells are equipped with several mechanisms that support their resistance to complement-dependent cytotoxicity (CDC). The role of the NF-κB pathway in cell protection from CDC was examined. Elevated sensitivity to CDC was demonstrated in cells lacking the p65 subunit of NF-κB or the IκB kinases IKKα or IKKβ, and in cells treated with p65 small interfering RNA. Pretreatment with the IKK inhibitor PS-1145 also enhanced CDC of wild-type cells (WT) but not of p65−/− cells. Furthermore, reconstitution of p65 into p65−/− cells and overexpression of p65 in WT cells lowered their sensitivity to CDC. The postulated effect of p65 on the JNK-mediated death-signaling pathway activated by complement was examined. p65 small interfering RNA enhanced CDC in WT cells but not in cells lacking JNK. JNK phosphorylation induced by complement was more pronounced in p65−/− cells than in WT cells. The results indicate that the NF-κB pathway mediates cell resistance to CDC, possibly by suppressing JNK-dependent programmed necrotic cell death.


Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Received for publication November 30, 2011. Accepted for publication May 8, 2012.

This work was supported by grants from the Israel Science Foundation and the Israel Cancer Association.

Address correspondence and reprint request to Prof. Zvi Fishelson, Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, P.O. Box 39040, Tel Aviv 69978, Israel. E-mail address: lifish@post.tau.ac.il

The online version of this article contains supplemental material.

Abbreviations used in this article: Bid, BH3 interacting domain death agonist; C5b-9, complement membrane attack complex composed of C5b, C6, C7, C8, and C9; CDC, complement-dependent cytotoxicity; CDE-HEPES, C9-deficient human serum; EGF, enhanced GFP; HIS, heat-inactivated normal human serum; IκK, IκB kinase; MAC, complement membrane attack complex; MEF, mouse embryonic fibroblast; MFI, mean fluorescence intensity; NHS, normal human serum; p65, RelA subunit of NF-κB; PI, propidium iodide; siRNA, small interfering RNA; SLO, streptolysin O; WT, wild-type.

Copyright © 2012 by The American Association of Immunologists, Inc. 0022-1767/12/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1103451

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1103451

The complement system plays a role in immune resistance to pathogenic microorganisms, facilitates immune complex clearance, and serves as an important link between innate and adaptive immune responses (1). Activated complement fragments and complexes can transmit stimulatory as well as inhibitory signals to many cell types (2–4). Cell damage is inflicted by complement upon assembly of the membrane attack protein complex (MAC) comprised of the C5b, C6, C7, C8, and C9 complement proteins (C5b-9) and its insertion into the plasma membrane of target cells (5). The membrane inserted C5b-9 complexes can transmit various signals to target cells (2). Activation of JNK and BH3 interacting domain death agonist (Bid) promotes cell death (6, 7). In contrast, activation of protein kinase C and ERK supports cell protection from complement-dependent cytotoxicity (8, 9). Cell resistance to complement-dependent cytotoxicity (CDC) also depends on the level of expression of the membrane complement regulatory proteins CD46, CD55, and CD59 (reviewed in Ref. 10) and the rate of elimination of the C5b-9 complexes from the cell surface (11, 12).

The NF-κB protein family participates in a wide range of physiological and pathological processes such as regulation of both innate and adaptive immune responses (13, 14), inflammation (15), and tissue development (16, 17). NF-κB links between inflammation and cancer and plays a role in tumor angiogenesis and invasiveness (18). The NF-κB family consists of five proteins: p105 (p50 when processed), p100 (p52 when processed), and the Rel subfamily consisting of RelA (p65), RelB, and c-Rel. These proteins form homo- or heterodimers that have distinct functions (19, 20). Through canonical and noncanonical signaling pathways, NF-κB regulates inflammatory and developmental processes, respectively. Upon activation of the IκB kinases IKKα or IKKβ, they phosphorylate IκBαs and target them to ubiquitination and proteasomal degradation, thus enabling translocation of the NF-κB homo- and heterodimers into the nucleus and activation of genes responsible for inflammation and survival (21–25).

NF-κB promotes cell survival by inhibiting the apoptotic machinery (26, 27). In the absence of NF-κB, persistent JNK activation is responsible for triggering cell death (28–30), suggesting antagonistic crosstalk between the NF-κB and JNK pathways. Our earlier results showed that JNK participates in MAC-induced signaling cascade leading to rapid necrotic cell damage (7). Results presented in this study demonstrate the contribution of the NF-κB pathway to cell resistance to complement-induced damage and propose involvement of a crosstalk between NF-κB and JNK in determination of cell fate following a complement cytotoxic attack.

Materials and Methods

Cells

Wild-type (WT), p65−/−, and IKKα−/− mouse embryonic fibroblasts (MEFs) prepared from WT, p65−/− (31), and IKKα−/− (32) knockout mice, respectively, were provided by Dr. Alexander Hoffmann (University of California, San Diego, La Jolla, CA). WT MEFs, IKKα knockout MEFs, IKKβ knockout MEFs, and IKKα−/− and IKKβ−/− double knockout MEFs (33, 34) were provided by Dr. Inder Verma (Salk Institute, San Diego, CA). HeLa and HEK 293T cells (American Type Culture Collection, Manassas, VA) and MEFs were maintained in DMEM supplemented with 10% heat-inactivated FBS (Life Technologies Laboratories, Grand Island, NY), 1% glutamine, 2% pyruvate, and an antibiotics mixture (Bio-Lab, Jerusalem, Israel) at 37°C and 5% CO2.

Sera, antisera, and reagents

Normal human serum (NHS) was prepared from healthy individuals and immediately frozen at −70°C until used. CS-deficient human serum (C8d-HS) was prepared from a CS-deficient patient. The serum served as a source for human complement and was not subjected to any analysis (hence an ethics approval was not required). Heat-inactivated NHS (HIS) was prepared by heating NHS at 56°C for 45 min. A polyclonal antiserum

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1103451

The Journal of Immunology

P.O. Box 39040, Tel Aviv 69978, Israel. E-mail address: lifish@post.tau.ac.il

The online version of this article contains supplemental material.

Abbreviations used in this article: Bid, BH3 interacting domain death agonist; C5b-9, complement membrane attack complex composed of C5b, C6, C7, C8, and C9; CDC, complement-dependent cytotoxicity; CDE-HEPES, C9-deficient human serum; EGF, enhanced GFP; HIS, heat-inactivated normal human serum; IκK, IκB kinase; MAC, complement membrane attack complex; MEF, mouse embryonic fibroblast; MFI, mean fluorescence intensity; NHS, normal human serum; p65, RelA subunit of NF-κB; PI, propidium iodide; siRNA, small interfering RNA; SLO, streptolysin O; WT, wild-type.

Copyright © 2012 by The American Association of Immunologists, Inc. 0022-1767/12/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1103451

The Journal of Immunology
directed to K562 cells was prepared by immunization of rabbits and was heat-inactivated as above. This antiserum contains anti-human cell Abs and is being used to activate human complement through the classical pathway on various human tumor and nontumoral cells, including HeLa and HEK 293T cells. Mouse anti-p–JNK and rabbit anti-p65 Abs were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-JNK Abs were purchased from Cell Signaling Technologies (Beverly, MA). Mouse anti-actin Abs were purchased from Chemicon (Temecula, CA). Abs directed to human C3 were prepared in a goat and were shown by Western blotting to bind primarily to C3 (α- and β-chains) in human serum. Mouse Abs directed to a neoepitope in human C5b-9 (clone e11) was purchased from Dako (Carpinteria, CA). The following Abs directed to human membrane complement regulatory proteins were used: anti-CD46 (clone GB24; provided by Dr. John Atkinson, St. Louis, MO), anti-CD55 purchased from Hycult Biotech (Uden, The Netherlands), and anti-hCD59 (clone BRIC229) purchased from the International Blood Group Reference Laboratory (Birmingham, U.K.). Anti-mouse CD59a was provided by Dr. Paul Morgan (University of Wales College of Medicine, Cardiff, U.K.). Monoclonal rat anti-mouse Cry was purchased from BD Biosciences. FITC-conjugated Abs directed to mouse, rabbit, goat, and rat IgG and peroxidase-conjugated goat anti-mouse IgG and goat anti-rabbit IgG were purchased from Sigma-Aldrich (St. Louis, MO).

**Cytotoxicity assays**

Cell lysis by complement (NHS) was measured by the PI and trypan blue inclusion methods as described before (6). Both methods measure plasma membrane damage and permeability and were used interchangeably. Briefly, human cells were treated with rabbit anti-K562 Abs for 30 min on ice followed by treatment with 50% NHS for 60 min at 37°C. Mouse cells were sensitive to 4–15% NHS and did not require pretreatment with DMSO. PS-1145 (an IKK inhibitor), propidium iodide (PI), streptolysin O (SLO), melittin, and DMSO were purchased from Sigma-Aldrich (St. Louis, MO).

**FIGURE 1.** Cells lacking p65 and IKK are more sensitive to CDC. (A and B) WT and p65−/− MEFs (A) or WT and IKKα−/− or IKKβ−/− MEFs (B) were treated with increasing concentrations of NHS for 60 min at 37°C. Percentage cell death was determined by trypan blue (A) or PI (B) inclusion. Results are of three independent experiments and are expressed as the mean percentage necrotic cell death ± SD, *p < 0.005 for p65−/−, IKKα−/−, or IKKβ−/− at all NHS concentrations relative to WT. (C) HeLa cells were transfected with 60 pmol p65 siRNA or with a nonspecific control siRNA. Cells were treated with Ab and NHS (50%) for 60 min at 37°C. Percentage cell death was determined by PI inclusion. Results of three independent experiments are expressed as the mean percentage lysis ± SD. Lysis of HIS-treated cells (3–7%) is not shown. *p < 0.01 relative to control siRNA. Right panel, Extracts of transfected and control HeLa cells were analyzed by Western blotting with rabbit anti-p65 Abs, using actin as a reference. (D) WT and p65−/− MEFs were pretreated with increasing concentrations of the IKK inhibitor PS-1145 or as control with PBS or DMSO for 60 min at 37°C and then treated with NHS (10% for WT cells and 5% for p65−/− cells) for 60 min at 37°C. Percentage necrotic cell death was determined by trypan blue inclusion. Results are of three independent experiments. *p < 0.05 relative to DMSO. NT, Not transfected.
Abs, probably due to presence of natural anti-mouse Abs in NHS. NHS lost its cytotoxicity on mouse cells after IgG depletion (M. Masarwa and Z. Fishelson, unpublished observations). Human complement was used to treat mouse fibroblasts since mouse complement has a lower cytotoxicity and is labile. Quantitative but not qualitative differences have been reported between cytotoxicity of C5b-9 complexes from various animal species. For the PI inclusion assay, PI was added to a final concentration of 0.5 μg/ml and EdTA to 1.5 mM. Cells were kept on ice and immediately examined in a FACScan (BD Biosciences). The data were analyzed by using WinMDI 2.8 and the percentage of dead (PI+) cells was calculated. Trypan blue inclusion was counted microscopically after addition of 0.2% trypan blue to the cells on ice. Statistical significance was analyzed by using the two-sided unpaired Student t test. All experiments included negative controls that tested the baseline death level. Death of control cells treated either without NHS (0% NHS) or with HIS (at the same concentration as NHS) was also determined.

**Lysis of cells by SLO and melittin**

HeLa cells (1 × 10^4) treated or not with p65 small interfering RNA (siRNA) for 2 d, as described below, were incubated for 10 min at 37°C with SLO and preactivated for 5 min at room temperature with 10 mM DTT or with DTT alone as control. FCS (final 5%) was added and incubation was continued for an additional 50 min at 37°C. Cells were also treated with the bee venom polypeptide melittin for 60 min at 37°C. The cells were then washed with PBS and treated with goat anti-mouse C3 antisera and mouse anti-neo C5b-9 (clone aE11) mAb for 30 min at 4°C, washed again, and labeled with FITC-conjugated secondary Abs. To determine the level of expression of complement regulators, mouse fibroblasts were treated with rabbit anti-mouse CDS9a (diluted 1:100) or rat anti-mouse Cry (diluted 1:150) and HeLa cells were treated with mouse anti-human CD46 (diluted 1:50), mouse anti-human CD55 (diluted 1:50), or mouse anti-human CDS9 (diluted 1:100) for 30 min at 4°C. After washing, the cells were labeled with FITC-conjugated secondary Abs. Labeled cells were examined by flow cytometry in a FACScan. Mean fluorescence intensity (MFI, G-mean) values for 7000 cells were analyzed by using WinMDI 2.8.

**Results**

**NF-κB protects cells from CDC**

NF-κB is a major regulator at the crossroad between life and death with known antiapoptotic activities (36). The role of NF-κB in complement-mediated cell death was studied first in MEFs lacking the p65 subunit of NF-κB (p65^-/-) or lacking the IKKα subunit or IKKβ subunit of the NF-κB activator complex (IKK). WT, p65^-/-, and IKK^-/- MEFs were reacted with complement and cell lysis was quantified. Compared to WT MEFs, p65^-/- MEFs, IKKα^-/- MEFs, and IKKβ^-/- MEFs were significantly more sensitive to complement-mediated necrotic cell death (Fig. 1A, B, C). The cells were then washed with PBS and treated with goat anti-human C3 antisera and mouse anti-neo C5b-9 (clone aE11) mAb for 30 min at 4°C, washed again, and labeled with FITC-conjugated secondary Abs. To determine the level of expression of complement regulators, mouse fibroblasts were treated with rabbit anti-mouse CDS9a (diluted 1:100) or rat anti-mouse Cry (diluted 1:150) and HeLa cells were treated with mouse anti-human CD46 (diluted 1:50), mouse anti-human CD55 (diluted 1:50), or mouse anti-human CDS9 (diluted 1:100) for 30 min at 4°C. After washing, the cells were labeled with FITC-conjugated secondary Abs. Labeled cells were examined by flow cytometry in a FACScan. Mean fluorescence intensity (MFI, G-mean) values for 7000 cells were analyzed by using WinMDI 2.8.
1B). Next, the effect of p65 silencing on complement-mediated death was examined in HeLa cells that underwent good transfection and silencing, thus also expanding the range of tested cells and excluding description of a cell-specific phenomenon. Cells were transfected with p65 siRNA or with a nonspecific control siRNA. Silencing efficiency of p65 siRNA was assessed 48 h after transfection by Western blotting using anti-p65 and anti-actin Abs (Fig 1C, right panel). Cells were also subjected to treatment with Ab and NHS and percentage cell death was determined by PI inclusion (Fig. 1C). Whereas control siRNA had no effect on HeLa complement sensitivity, compared with untransfected cells, p65 silencing sensitized cells to complement-mediated necrotic cell death.

Cells lacking the IKKα or IKKβ subunit of the NF-κB activator complex are more sensitive to CDC than are WT cells (Fig. 1B), suggesting involvement of IKK in complement resistance. PS-1145 specifically blocks NF-κB activation by inhibiting phosphorylation of IκBα, the key negative regulator of p65 activity, by IKK (37). The effect of short-term inhibition of the NF-κB pathway with PS-1145 on CDC was examined. Pretreatment with PS-1145 enhanced complement-mediated cell death of WT MEFs (Fig. 1D). In contrast, PS-1145 had no effect on death of p65−/− MEFs, further supporting a role for IKK and the p65 pathway in cell resistance to CDC.

Complement-mediated death of p65−/− MEFs reconstituted with plasmid p65 was examined. p65−/− MEFs were transiently transfected with increasing quantities of pEGFP-p65 plasmid (1–3 μg). As shown in Fig. 2A, increasing doses of recombinant p65-EGFP were synthesized by the transfected cells. Restoration of p65 in p65−/− cells correlated with a dose-dependent reduction of sensitivity to complement-mediated cell death (Fig. 2B). Next, the effect of p65 overexpression in HEK 293T cells on CDC was examined. HEK 293T cells were transiently transfected with pEGFP-p65 or with control vector. Overexpression of p65-EGFP significantly reduced death of HEK 293T cells by complement (Fig. 2C). Interestingly, treatment of transfected cells with sublytic complement had no apparent effect on cellular location of EGFP-p65 whereas treatment with TNF-α induced a rapid nuclear translocation of EGFP-p65 (Supplemental Fig. 1).

**NF-κB does not protect cells from other pore formers**

To examine whether NF-κB protects cells from pore formers in general or is selective to C5b-9, the impact of p65 silencing on cell death inflicted by the pore formers SLO and melittin was tested. SLO and melittin use a similar mechanism to that of complement MAC to incorporate into cell membranes, induce Ca2+ influx, and produce cell death (38). HeLa cells were transfected, using Oligofectamine, with p65 siRNA or with a nonspecific control siRNA. Forty-eight hours after transfection, untransfected and transfected cells were treated with SLO preactivated with DTT or DTT alone as control or with melittin. As shown in Fig. 3, p65 silencing by siRNA had no effect on cell death mediated by melittin (Fig. 3A) or SLO (Fig. 3B).
Effect of NF-κB on complement deposition

NF-κB regulates transcription of numerous target genes (39). Therefore, NF-κB knockdown could potentially modify cell surface proteins, leading to enhanced complement activation and increased deposition of complement proteins on the cells. The extent of complement C5b-9 deposition and cell death does not correlate well with quantity of Ab binding, but it correlates excellenty with the level of expression of the membrane complement regulators (Ref. 12 and Z. Fishelson, unpublished data). Thus, for example, a decrease in the level of expression of the membrane complement regulator CD59 may lead to enhanced complement deposition and cell death. The level of expression of murine Crry and CD59a on WT and p65−/− MEFs was measured by flow cytometry, as described in Materials and Methods. The levels of Crry and CD59 on p65−/− were somewhat lower than on WT MEFs (Fig. 4A). Analysis of the level of expression of CD46, CD55, and CD59 on HeLa cells transfected with p65 siRNA or control siRNA showed no difference in expression of these regulatory proteins (Fig. 4C). The relative capacity of WT and p65−/− cells to activate complement was also compared. WT and p65−/− MEFs were treated with C8D-HS for C3 deposition or NHS for C5b-9 deposition as described in Materials and Methods. Cells were washed and labeled with anti-C3 or anti–C5b-9 aE11 Abs followed by FITC-labeled second Abs. The lower membrane regulator expression in the absence of p65 had no impact on C3 and C5b-9 deposition on MEFs (Fig. 4B). Similarly, HeLa cells transfected with p65 siRNA or control siRNA were treated with Ab and NHS and analyzed for C3 and C5b-9 deposition. C3 deposition was not affected by p65 silencing whereas a small increase of MAC deposition was observed on HeLa cells transfected with p65 siRNA relative to control siRNA (Fig. 4D).

Is NF-κB targeting JNK in its protective activity from complement?

JNK activation is involved in cell death induced by complement (7). To examine the hypothesis that NF-κB protects cells from complement by downregulating the JNK pathway, the effect of JNK silencing on death of cells lacking p65 was investigated. HeLa cells were transfected with p65 siRNA, JNK siRNA, or both. Silencing of p65 and JNK was validated 48 h after transfection by Western blotting, using anti-p65, anti-JNK, and anti-actin Abs (Fig. 5B). At the same time, these transfecants were subjected to CDC by Ab and NHS. As shown in Fig. 5A and as expected, p65 silencing enhanced cell death whereas JNK silencing reduced cell death. Interestingly, p65 silencing lost its enhancing effect in JNK− cells. This suggests that p65 has no protective effect in the absence of JNK.

To further demonstrate an effect of NF-κB on JNK activation, the kinetics of complement-induced JNK activation were examined in WT and p65−/− MEFs. Cells were treated with NHS or HIS for 10–30 min at 37˚C and cell extracts were subjected to Western blotting with anti–p-JNK or anti-actin Abs. HIS induced in both cell lines low-level JNK activation, probably due to serum effects (Fig. 5C, 5D). In contrast, following sublytic complement (NHS) treatment, JNK activation was significantly higher in p65−/− MEFs (Fig. 5D) than in WT MEFs (Fig. 5C).

FIGURE 5. Crosstalk between p65 and JNK in regulation of CDC. (A and B) HeLa cells were transfected with p65 siRNA, JNK siRNA, with both siRNAs, or with a nonspecific control siRNA. Forty-eight hours after transfection, the cells were treated with Ab and NHS (50%) for 60 min at 37˚C. Percentage necrotic cell death was determined by trypan blue inclusion. Results of three experiments are expressed as the mean percentage cell death ± SD. *p < 0.05 relative to control siRNA. (B) Transfected HeLa cells in (A) were extracted and examined by Western blotting with anti-p65, anti-JNK, or anti-actin Abs. Anti-JNK Ab detects both p54 and p46 JNK isoforms. (C and D) WT (C) and p65−/− (D) MEFs were treated with 7% NHS or HIS at 37˚C for the indicated times. Cell extracts were subjected to Western blotting with anti–p-JNK (and anti-actin) Abs. Band intensities of the p54 JNK isoform were quantified by densitometry and corrected according to the intensity of the actin band. In three independent experiments, the corrected optical densities of the p-JNK bands were adjusted relative to the p-JNK band of HIS-treated cells at the 10 min point, which was set as 100. The mean adjusted p-JNK band intensities of the three experiments ± SD are shown. *p < 0.05 relative to HIS.
Discussion

The cell death and survival signals that activate and regulate complement-dependent cytotoxicity mediated by the complement C5b-9 complex are not well characterized. At least to some extent, the necrotic cell death inflicted by the C5b-9 complex is programmed and involves Bid and JNK (6, 7). Some of the death signals are counteracted by ERK (9). Results presented in this study demonstrate that the NF-κB signaling pathway also participates in cell protection from complement-mediated cell death. This claim is supported by several findings: 1) cells lacking either the p65 subunit of NF-κB or the IKKα or IKKβ subunits of the NF-κB activator complex are more sensitive to CDC; 2) knockdown of p65 with siRNA renders cells more susceptible to CDC; 3) pretreatment of target cells with the IKK inhibitor PS-1145 increases their sensitivity to CDC, but only in p65-deficient cells; and 4) reconstitution of p65 into p65-deficient and p65-sufficient cells enhances their resistance to CDC. Apparently, both p65 and IKKβ of the canonical NF-κB pathway and IKKα of the noncanonical NF-κB pathway are involved in the protection from CDC. One possibility is that both canonical and noncanonical NF-κB pathways promote resistance to CDC. Alternatively, the canonical and noncanonical NF-κB signaling pathways may cross-regulate each other and act as a multifactorial and depends, besides the membrane complement regulators, on soluble complement regulators such as factor H and C4b binding protein, on cell surface sialic acid residues, and on several cellular mechanisms (reviewed in Ref. 10), this is not surprising. Bid is involved in necrotic cell death activated by both C5b-9 and SLO (6). Unlike C5b-9, SLO does not induce JNK activation (7). Hence, it is suggested that JNK activation is involved in CDC but not in cell death induced by SLO or melittin. Consequently, the NF-κB p65-dependent protective mechanism targeting JNK would be effective against CDC but not against cell death induced by SLO and melittin. Furthermore, C5b-9 activates necrotic cell death via JNK-dependent and JNK-independent pathways (7). That knockdown of p65 has no effect on JNK cells (Fig. 5A) suggests that p65 has no protective effect in the JNK-independent lytic pathway activated by C5b-9.

Acknowledgments

p65−/− and IKKα−/− mouse embryonic fibroblasts were provided by Dr. Alexander Hoffmann (University of California, San Diego), and pEGFP-p65 was provided by Dr. Lienhard Schmitz (University of Giessen, Germany). IKKα−/− MEFs and IKKβ−/− MEFS were provided by Dr. Inder Verma (Salk Institute, La Jolla, CA). Anti-mouse CD59a was provided by Dr. Paul Morgan (University of Wales College of Medicine, Cardiff, U.K.).

Disclosures

The authors have no financial conflicts of interest.

References


