Role of TLR-2 in the Activation of Nuclear Factor κB by Oxidative Stress in Cardiac Myocytes*

Received for publication, October 6, 2000, and in revised form, November 15, 2000
Published, JBC Papers in Press, November 16, 2000, DOI 10.1074/jbc.M009160200

Stefan Frantz, Ralph A. Kelly, and Todd Bourcier‡
From the Cardiovascular Division, Brigham and Women’s Hospital, Boston, Massachusetts 02115

Growing evidence from patients with heart failure and from experimental animal models implicates effectors of innate immunity in the pathogenesis of this syndrome. The expression of the innate immunity signaling protein, Toll-like receptor 4 (TLR4), is increased in cardiac myocytes in situ and in failing myocardium, but the mechanism by which TLRs may be activated in the failing heart remains unclear. We report that TLR2, which is expressed in cardiac myocytes, participates in the response of these cells to oxidative stress, a major contributor to the pathogenesis of cardiac dysfunction. Hydrogen peroxide increased nuclear factor κB (NF-κB) activation in Chinese hamster ovary fibroblasts that overexpress TLR2 but not in normal or TLR4-overexpressing Chinese hamster ovary cells, an effect that was abrogated by an α-TLR2 antibody. In neonatal rat ventricular myocytes, the α-TLR2 antibody inhibited hydrogen peroxide-induced nuclear translocation of NF-κB and activator protein-1 (AP-1). Inhibition of TLR2 had no effect on tumor necrosis factor α-induced NF-κB or AP-1 activation, on the DNA binding of the basal transcription factor Oct-1, or on hydrogen peroxide-induced phosphorylation of p38 MAP kinase. Importantly, oxidative stress-induced cytotoxicity was enhanced by blocking TLR2. Given the importance of cytotoxicity and apoptosis to the pathology of the ischemic heart, an anti-apoptotic effect of TLR2 in cardiac myocytes exposed to elevated levels of ROS may limit further cardiac dysfunction.

The mammalian toll-like receptors (TLRs)1 are a recently recognized family of proteins implicated in directing innate immune responses by components of diverse pathogens (1, 2). So far, six human TLRs have been cloned (3, 4), and several others are suggested to be present. Medzhitov et al. (5) were the first to show that constitutively active TLR4 induces the activation of NF-κB with the subsequent expression of IL-1, IL-6, IL-8, and the costimulatory molecule B7.1. Active TLR4 can also induce c-Jun NH2-terminal kinase (JNK) (6). The role of TLRs as innate immunity receptors has been extensively studied. TLR2, for example, is activated by components of Gram-positive bacteria (7), mycobacteria (8, 9), and Borrelia (10). The most convincing evidence exists for a role of TLR4 in the recognition of bacterial LPS (lipopolysaccharides). Mice with either a spontaneous mutation of the TLR4 gene or targeted disruption of the gene have no response to lipopolysaccharides and are thus resistant to endotoxic shock (11–13). In contrast, mice with targeted disruption of TLR2 have no deficit in the recognition of Gram-negative bacteria (14). Interestingly, missense mutations affecting the extracellular domain of the TLR4 receptor are associated with a blunted response to inhaled LPS in humans (15).

Most studies have focused on TLR expression and function in mononuclear cells and monocyte-like cell lines; however, TLRs differentially exhibit a wider pattern of tissue expression, including the lung, heart, and brain (2). Thus, TLRs may additionally serve as pro-inflammatory receptors in cell types without a dedicated immune function. TLR4, for example, is expressed by human dermal endothelial cells and can activate NF-κB (16). We previously demonstrated (17) that TLR4 is expressed in the heart and that injured human and murine myocardium exhibit focal areas of intense TLR4 expression. The reason for this differential expression of TLR4, as well as the function of TLR4 in the injured heart, in the absence of infection, remains unknown. Although the identity of endogenous ligands, analogous to Drosophila spatzle, for the vertebrate TLRs remains elusive, a recent report suggests that the stress-associated factor heat-shock protein 60 signals through TLR4 (18). Thus, innate immunity receptors may not only be activated by microorganisms, but also by endogenous signals that originate from injured cells that emanate “danger signals” (19).

Oxidative stress in the post-ischemic heart has been identified as an important event in myocardial dysfunction, linked to both myocardial hypertrophy and apoptosis. Oxidative stress is known to induce cell death by apoptotic and necrotic pathways in isolated cardiac myocytes (20) and to trigger pro-inflammatory signaling pathways that activate NF-κB and AP-1 transcription factors (21, 22). Numerous mechanisms are suggested to underlie these effects of hydrogen peroxide, which are likely to be cell type-specific. Herein, we show a requirement of TLR2 for the activation of NF-κB and AP-1 by hydrogen peroxide and provide evidence that TLR2 imparts an anti-apoptotic effect in stressed or injured cardiac myocytes.

MATERIALS AND METHODS

Chemicals—Human recombinant TNFα was purchased from Endogen (Woburn, MA). All other chemicals, including hydrogen peroxide, were purchased from Sigma Chemical Co. unless noted otherwise.
Cell Lines, Cell Isolation, and Culture—Chinese hamster ovary K1 (CHO-K1) fibroblasts were obtained from D. T. Golenbock (23). The cell lines stably express CD-14, an NF-κB-dependent CD25 reporter construct, and empty vector (CHO/control), human TLR2 (CHO/TLR2), or human TLR4 (CHO/TLR4). The cells were maintained as described (23). Cells were stimulated as indicated in Ham’s F-12 medium containing 10% fetal calf serum (Life Technologies, Inc.).

Neonatal rat ventricular myocytes (NRVM) were isolated from 1-day-old Harlan Sprague-Dawley pups as described (17). NRVM were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum (Life Technologies, Inc.). After 48 h, the medium was changed to Dulbecco’s modified Eagle’s medium (F-12 medium (Life Technologies, Inc.) containing 1% insulin, transferrin, selenium media supplement (ITS, Sigma) with antibiotics. Treatments were initiated 12 h later.

CHO fibroblasts and NRVM were pretreated with a TLR-2 blocking antibody raised to a peptide that maps to the amino terminus of human TLR2 (6 or 10 μg/ml, number N-17, Santa Cruz Biotechnologies Inc., Santa Cruz, CA) for 1 h prior to addition of experimental treatments.

Western Blotting—For Western blots, cells were harvested in SDS sample buffer (62.5 mM Tris base, 2% SDS, 10% glycerol, 50 mM dithiobisulfoxide and 1% 2-mercaptoethanol). The cell pellets were washed with PBS, cells were boiled, equal amounts of the denatured protein per lane were loaded, separated on 7.5 or 15% SDS-polyacrylamide gel electrophoresis, and transferred to polyvinylidene fluoride membranes. After blocking with 5% normal horse serum (Vector Laboratories, Burlingame, CA), the membranes were incubated with the primary antibody, an anti-goat FITC-conjugated secondary antibody, a secondary Alexa 568-tagged anti-mouse antibody (Molecular Probes, Eugene, OR). Immunoblotting was performed as described in the manufacturer’s protocol. cDNA reactions for AP-1 activity contained an additional 5 mM MgCl2. Control reactions contained 100-fold excess of unlabeled oligonucleotide and were incubated with nuclear extracts as indicated. DNA complexes were separated on a 5% non-denaturing polyacrylamide gel in 0.5× TBE buffer. The DNA in the gels was transferred to nitrocellulose membranes, which were incubated for 1 h with a matching secondary antibody, a second primary rat caveolin 3 antibody (Transduction Laboratories, Lexington, KY), and a secondary Alexa 568-tagged anti-mouse antibody (Molecular Probes, Eugene, OR). Images were taken with a confocal laser scanning microscope (model MRC-1024, Bio-Rad Laboratories, Hercules, CA). The specificity of the primary goat polyclonal anti-hTLR2 antibody was assessed by preincubating the antibody with the hTLR2 matching peptide (Santa Cruz Biotechnologies Inc.).

Flow Cytometry Analysis of NF-κB Activity—CHO cells were stably transfected with a luciferase reporter (38) and labeled with an FITC CD25 monoclonal antibody (Becton Dickinson Immunocytochemistry Systems, San Jose, CA). Analysis was performed on 10,000 cells/sample using a FACSscan.

Autoradiographic Assay—Cells were treated as indicated for 2 h following pretreatment with the blocking antibody for 1 h. Electromobility shift assays were performed as described elsewhere (24). Binding reactions were performed with 10 μg of nuclear protein. The binding reactions for AP-1 activity contained an additional 5 mM MgCl2. Control reaction mixtures contained a 100-fold excess of unlabeled oligonucleotide and were incubated with nuclear extracts as indicated. DNA complexes were separated on a 5% non-denaturing polyacrylamide gel in 0.5× Tris-borate EDTA buffer.

Tris-borate EDTA buffer. NF-κB, AP-1, and Oct-1 oligonucleotides were purchased from Santa Cruz Biotechnologies.

Flow Cytometry Analysis for the Detection of Sub-G1 and Phosphatidylserine—FACS was used to detect cells with a hypodiploid content of DNA and surface expression of phosphatidylserine by staining with FITC-annexin V. NRVM were collected by trypsinization, pooled with nonattached cells, and fixed with ice-cold 70% ethanol/PBS. After being rinsed with PBS, cells were incubated with a propidium iodide (20 μg/ml) solution containing RNase A (5 Kunitz units/ml) at room temperatur70°C.
temperature for 30 min. NRVM in the sub-G₁ region were identified using ModFit software, and data are expressed as the percentage of cells in sub-G₁. For detection of surface phosphatidylserine, NRVM were collected by trypsinization and directly stained with FITC-annexin V, according to the manufacturer’s direction (CLONTECH, Palo Alto, CA). FACS analysis was performed on 10,000 cells/sample using a FACscan cytometer (Becton Dickinson).

Statistical Analyses—All replicate data are expressed as mean and standard error of mean. In experiments with comparison of treatments, an unpaired Student’s t test was performed. In experiments with time courses, analysis of variance was used followed by Fisher’s post-hoc test. Statistical significance was achieved when \( p \leq 0.05 \). Statistical analyses were carried out using StatView statistics program (Abacus Concepts, Inc., Berkley, CA).

RESULTS

Expression Pattern of TLRs in Neonatal Rat Cardiac Myocytes—Six human TLR cDNAs have been identified (3, 4). However, their distribution in cardiac tissue is unknown. TLR2, TLR3, TLR4, and TLR6 were detectable by reverse transcription-PCR in primary NRVM cultures (Fig. 1a). Signals for TLR1 and TLR5 were absent in cardiac tissue, whereas spleen showed the expected TLR1 and TLR5 bands (data not shown).

We previously reported that TLR4 protein is expressed by cardiac myocytes (17) but did not explore the expression of TLR2. Using a polyclonal TLR2 antibody, a single band was detected in a TLR2-overexpressing cell line (CHO/TLR2) but not in control (CHO/control) or TLR4-overexpressing (CHO/TLR4) cell lines. A corresponding band could be detected in lysates of neonatal cardiac myocytes (Fig. 1b). Immunocytochemical staining with the same antibody confirmed the expression of TLR2 in myocytes (Fig. 1c). Primary isolates of neonatal rat ventricular myocytes in vitro exhibited sarcolemmal membrane staining that was inhibited by preincubation with the corresponding TLR2 peptide used to generate the primary antibody. That TLR2-positive cells also expressed caveolin 3 verified their identity as cardiac myocytes.

**TLR2 Enhances NF-κB Activity after Stimulation with Hydrogen Peroxide in a CHO Cell Line**—Reactive oxygen intermediates play a key role in cardiac damage following ischemic injury. To investigate a potential role of TLRs in the response...
to oxidative stress, TLR-expressing CHO cell lines were used. CHO fibroblasts do not express mRNA for full-length and functional TLR2 (25). The obtained cell lines have previously been characterized (23) and overexpress CD14, inducible membrane CD25 under the control of an NF-κB promoter, and empty vector (CHO/control), human TLR2 (CHO/TLR2), or human TLR4 (CHO/TLR4). Oxidative stress was induced by the application of hydrogen peroxide (1 mM). The activity of NF-κB could be monitored by expression of membrane CD25 by flow cytometric analysis. Untreated cells showed nearly equal expression of CD25. TNFα increased CD25 expression evenly in the CHO/control, CHO/TLR2, and CHO/TLR4 lines after 24 h of treatment. However, application of hydrogen peroxide consistently increased NF-κB activity in the CHO/TLR2 cells to a greater extent than in the CHO/control and CHO/TLR4 cell lines (Fig. 2a). In addition, hydrogen peroxide increased nuclear translocation of NF-κB in the CHO/TLR2 line, but was ineffective in the CHO/control line after 2 h of treatment, as measured by EMSA. There was no difference in the amount of nuclear NF-κB for TNFα-treated cells (Fig. 2b). Moreover, a 1-h preincubation with a TLR2 blocking antibody but not control IgG (data not shown) completely abrogated the nuclear translocation of NF-κB. The binding of Oct-1, a transcription factor that is constitutively expressed, was unchanged by the treatment (Fig. 2c). CHO cells showed a high basal level of nuclear AP-1 by EMSA without treatment, and was unchanged by preincubation with the TLR2 antibody or treatment with hydrogen peroxide (data not shown). These results suggest that TLR2 enhances hydrogen peroxide-induced activation of NF-κB.

**TLR2 Is Involved in Nuclear Translocation of NF-κB after Oxidative Stress in Neonatal Rat Cardiac Myocytes**—To test whether TLR2 participates in the response to oxidative stress in cardiac myocytes, NRVM were preincubated with the blocking TLR2 antibody for 1 h and then treated with hydrogen peroxide for another 2 h. As for the CHO cells, the TLR2 blocking antibody could completely abrogate the nuclear translocation of NF-κB (Fig. 3a), whereas preincubation with control IgG was without effect. NF-κB activation by TNFα was unchanged by treatment with the TLR2 blocking antibody. Similar to NF-κB, AP-1 induction by hydrogen peroxide could be blocked by the TLR2 blocking antibody, but not AP-1 induction by TNF-α. The constitutively expressed Oct-1 was unchanged by the treatments.

Oxidative stress is also known to rapidly induce the phosphorylation of p38 MAPK in cardiac myocytes (26). However, blocking of TLR2 had no effect on hydrogen peroxide-induced p38 phosphorylation after 15 min of treatment (Fig. 3b).

**Fig. 3.** TLR2 is involved in hydrogen peroxide-induced NF-κB activation in neonatal rat ventricular myocytes. a, pretreatment with a TLR2 blocking antibody inhibited nuclear translocation of NF-κB and AP-1 after a 2-h treatment with hydrogen peroxide (left panels) but had no influence on the constitutively expressed transcription factor Oct-1 or on nuclear translocation of NF-κB after treatment with TNF (middle panels). A nonimmune IgG antibody (NI IgG) did not affect hydrogen peroxide-induced NF-κB translocation (right panel). b, hydrogen peroxide-induced phosphorylation of p38 MAP kinase, shown in the top panel using an antibody specific for phosphorylated p38 MAPK, is unaffected by the anti-TLR2 blocking antibody. The lower panel shows equal amounts of p38 MAPK in each lane. All experiments were done in triplicate.
An Anti-apoptotic Role of TLR2 after Oxidative Stress in Cardiac Myocytes—NF-κB activation can elicit anti-apoptotic and pro-apoptotic pathways in various cell types. Whether NF-κB promotes or inhibits apoptosis seems dependent on the type of cell and the type of inducer (27). Under the conditions of ischemic injury and oxidative stress, NF-κB appears to have an anti-apoptotic role in cardiac myocytes (28). To investigate the role of TLR2 in myocyte survival after oxidative stress, NRVM were preincubated with a blocking TLR2 antibody for 1 h and then treated with 0.5 mM hydrogen peroxide. The fraction of myocytes with DNA fragmentation, measured as the percentage of cells in sub-G1 phase, ranged from 6.9 to 10.9% under basal, serum-free conditions over the 6-h incubation. Hydrogen peroxide increased this number to 12 ± 4.6% at 30 min, to a maximum of 33.2 ± 5.9% at 6 h. Preincubation with the TLR2 antibody further increased hydrogen peroxide-induced DNA fragmentation at all time points examined, to a maximum of 40.5 ± 4.9% at 6 h. A nonimmune IgG was without effect (Fig. 4a) on the response to hydrogen peroxide.

Annexin V has a strong, specific affinity for phosphatidylserine (PS). Shortly after cells are induced into apoptosis, PS redistributes from the inner to the outer layer of the cell membrane, thus providing a readily detectable marker of dying cells (29). Basal levels of annexin V-positive cells in these experiments were found to be high, averaging ~25%. Hydrogen peroxide stimulation had no effect at 30 min but modestly increased annexin V staining of NRVM after 2 h incubation, to 35%. As shown in Fig. 4b, blockade of TLR2 increased the response to hydrogen peroxide at 30 min (H$_2$O$_2$, 25% versus H$_2$O$_2$+α-TLR2 Ab, 65%), and at 2 h (H$_2$O$_2$, 35% versus H$_2$O$_2$+α-TLR2 Ab, 46%). Pretreatment with a nonimmune IgG was without effect. Thus, blockade of TLR2 inhibits hydrogen peroxide-induced NF-κB and AP-1 activity but enhances hydrogen peroxide-induced DNA fragmentation and PS externalization in NRVM, suggesting an anti-apoptotic function of TLR2 in cardiac myocytes after oxidative stress.

**DISCUSSION**

The innate immunity system is an evolutionarily ancient and conserved form of the immune system that is triggered by soluble and membrane bound “pattern recognition receptors” (PRR). In the expanded self-nonself model of innate immunity, PRRs have evolved to distinguish between dangerous and innocuous nonself antigens. Therefore, innate immunity recognizes invariant patterns (i.e. pathogen-associated molecular patterns) shared by groups of microorganisms but not by host tissues (30, 31). A typical pathogen-associated molecular pattern would be bacterial lipopolysaccharides (LPS), for example. However, Matzinger (19, 32) has suggested a different explanation for function and triggers of the innate immune system. In the “Danger” model, the immune system would be activated by endogenous “alarm” or “danger” signals, which originate from stressed, injured, or necrotic cells and not by highly conserved structured motifs in pathogens. These danger signals are recognized by the pattern recognition receptors and activate the innate immune system. Therefore, PRRs of the innate immune system would recognize specific epitopes or ligands that indicate injured, dying, or dead cells (i.e. injured/dead self) even in the absence of infection. Signals of “stressed” cells could be sensed by changes in the lipid and/or carbohydrate moieties expressed on the surface of cells or by the presence of proteins not normally found in the outer cell membrane.

Increased myocardial expression of inflammatory cytokines, such as TNF, IL-1β, and IL-6, occurs in human and experimental heart failure, seemingly irrespective of initiating events and without evidence of infection (33). Although there is a growing consensus that one or more of these proteins contribute to maladaptive cardiac remodeling, the proximal events that trigger and sustain their expression are not well understood. However, each of these cytokines is now known to be related to innate immunity, and their expression may be initiated through activation of specific innate immunity receptors. We have previously shown that TLR4 is expressed in cardiac myocytes and that overexpression of dominant positive TLR4 activates NF-κB. In normal murine and human myocardium, TLR4 expression was diffuse and predominantly expressed in cardiac myocytes. However, in both “remodeling” murine and human myocardium remote from sites of ischemic injury and in heart tissue from patients with idiopathic congestive heart failure, focal areas of intense TLR4 staining in myocytes could be
demonstrated that endogenous heat shock protein 60 (HSP60) outlined by Matzinger (19). In support of this, a recent report "alarm" or "danger" signals that activate innate immunity as can modify membrane components and is a known inducer a classical ligand/receptor complex. However, hydrogen perox-

s. Frantz, R. A. Kelly, and T. Bourcier, unpublished observations.

REFERENCES

1. Medzhitov, R., and Janeway, C., Jr. (2000) N. Engl. J. Med. 343, 338–344
2. Schuster, J. M., and Nelson, P. S. (2000) J. Leukoc. Biol. 67, 767–773
3. Bastuji, F. L., Hardiman, G., Panagos, J. C., Kastner, R. A., and Bazan, J. F. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 588–593
4. Takeuchi, O., Kawai, T., Sanjo, H., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Takeda, K., and Akira, S. (1999) Gene 231, 59–65
5. Medzhitov, R., Preston-Hurlbut, P., and Janeway, C. A., Jr. (1997) Nature 388, 394–397
6. Muzio, M., Nafisi, G., Sacchi, S., Leverero, M., and Mantovani, A. (1998) J. Exp. Med. 187, 2097–2102
7. Schwandner, R., Dzierski, R., Wescott, W., Roth, M., and Kirschning, C. J. (1999) J. Biol. Chem. 274, 17406–17419
8. Brightbill, H. D., Libraty, D. H., Kutzik, S. R., Yang, R. B., Belsie, J. T., Bleiharski, J. R., Maltland, M., Norgard, M. V., Plevy, S. E., Smale, S. T., Brennan, P. J., Bloom, B. G., Godowski, P. J., and Modlin, R. L. (1999) Science 285, 732–736
9. Underhill, D. M., Ozinsky, A., Smith, K. D., and Aderem, A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 14459–14463
10. Hirschfeld, M., Kirschning, C. J., Schandauer, R., Wescott, W., Heis, J. H., and Modlin, R. L. (1999) J. Immunol. 163, 2392–2396
11. Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., Takeda, K., and Akira, S. (1999) J. Immunol. 162, 3749–3752
12. Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Huflej, C. V., Du, X., Birdwell, D., Alavez, E., Silva, M., Galanis, C., Feudenberg, M., Ricciardi-Castagnoli, P., Layton, B., and Beutler, B. (1998) Science 282, 2985–2988
13. Qureshi, S. T., Lariviere, L., Leveque, G., Clermont, S., Moore, K. J., Gros, P., and Mado, D. (1999) J. Exp. Med. 189, 615–625
14. Takeuchi, O., Hoshino, K., Kawai, T., Sanjo, H., Takada, Y., and Akira, S. (1999) Immunity 11, 443–451
15. Armour, N. C., Lorenz, E., Kell, R. C., Zahn, J., Kline, J. N., Jones, M., Frels, R., Wirt, J. L., and Schwartz, D. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 187–191
16. Zhang, F. X., Hoshino, K., Takeda, K., and Akira, S. (1999) J. Clin. Invest. 104, 1871–1878
17. Frantz, S., Koblik, L., Kim, Y. D., Fukazawa, R., Medzhitov, R., Lee, R. T., and Kelly, R. A. (1999) J. Clin. Invest. 104, 271–280
18. Obashi, K., Burkart, V., Klebe, S., and Kolb, H. (1999) J. Immunol. 163, 558–561
19. Matzinger, P. (1999) Semin. Immunol. 11, 399–415
20. von Hardestorf, B., Li, P. F., and Dietz, R. (1999) Circulation 99, 2934–2941
21. Bowie, A., and O'Neill, L. A. (2000) Biochem. Pharmacol. 59, 13–23
22. Peng, M., Huang, L., Xie, J. Z., Huang, W. H., and Askari, A. (1999) Cell Mol. Biol. Res. 41, 189–197
23. Lien, E., Sellati, T. J., Yoshimura, A., Foi, T. H., Rawadi, G., Finch, R. W., Caroll, J. D., Espevik, T., Ingalls, R., Radolf, J. D., and Golenbock, D. T. (1999) J. Biol. Chem. 274, 33419–33425
24. Bourcier, T. (1999) in Methods in Molecular Medicine, Vascular Disease (Baker, A. H., ed) pp. 159–167, Humana Press, Totowa, NJ
25. Heine, H., Kirschning, C., Lien, E., Monks, B., Roth, M., and Golenbock, D. N. (1999) J. Immunol. 162, 6971–6975
26. Guyton, K. Z., Liu, Y., Gorospe, M., Xu, Q., and Holbrook, N. J. (1999) J. Biol. Chem. 271, 4138–4142
27. Barkett, M., and Gilmore, T. D. (1999) Oncogene 18, 6910–6924
28. Mustapha, S., Kirshner, A., De Meissac, D., and Kirchenbaum, L. A. (2000)
TLR-2 in Activation of NF-κB by Oxidative Stress

29. Martin, S. J., Reutelingsperger, C. P., McGahan, A. J., Rader, J. A., van Schie, E. C., LaFace, D. M., and Green, D. R. (1995) J. Exp. Med. 182, 1545–1556
30. Janeway, C. A., Jr. (1992) Immunol. Today 13, 11–16
31. Medzhitov, R., and Janeway, C. A., Jr. (1997) Cell 91, 295–298
32. Matzinger, P. (1994) Annu. Rev. Immunol. 12, 991–1045
33. Medzhitov, R., and Janeway, C. A., Jr. (1997) Cell 91, 295–298
34. Matzinger, P. (1994) Annu. Rev. Immunol. 12, 991–1045
35. Medzhitov, R., and Janeway, C. A., Jr. (1997) Cell 91, 295–298
36. Matzinger, P. (1994) Annu. Rev. Immunol. 12, 991–1045
37. Turner, N. A., Xia, F., Azhar, G., Zhang, X., Liu, L., and Wei, J. Y. (1998) J. Mol. Cell Cardiol. 30, 1789–1801
38. Kol, A., Bourcier, T., Lichtman, A. H., and Libby, P. (1999) J. Clin. Invest. 103, 571–577
39. Aliprantis, A. O., Yang, R. B., Mark, M. R., Suggest, S., Devaux, B., Radolf, J. D., Klimpel, G. R., Godowski, P., and Zychlinsky, A. (1999) Science 285, 736–739
40. Aliprantis, A. O., Yang, R. B., Weiss, D. S., Godowski, P., and Zychlinsky, A. (2000) EMBO J. 19, 3325–3336
41. Anversa, P., Leri, A., Beltrami, C. A., Guerra, S., and Kajstura, J. (1999) Lab. Invest. 79, 111–129
42. Haunstetter, A., and Ixamo, S. (1998) Circ. Res. 82, 111–129