CD14-dependent Lipopolysaccharide-induced β-Defensin-2 Expression in Human Tracheobronchial Epithelium*

Received for publication, January 10, 2000, and in revised form, June 30, 2000

Marie N. Becker‡, Gill Diamond§, Margrith W. Verghesee‡, and Scott H. Randell‡†

From the ‡Cystic Fibrosis/Pulmonary Research and Treatment Center, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599 and the §Department of Anatomy, Cell Biology and Injury Sciences, UMDNJ-New Jersey Medical School, Newark, New Jersey 07103

The innate immune response comprises the first line of host defense against pathogenic microorganisms. Many protective elements including nitric oxide, peptides/proteins, and whole cells such as phagocytes and natural killer cells have been conserved during evolution. Studies in Drosophila illustrate the primary importance of pathogen recognition and induction of antimicrobial molecules. Toll mutations preventing antifungal peptide induction render mutant flies susceptible to fungal infection (1). Signaling through Drosophila Toll results in activation of members of the NF-κB family of transcription factors and expression of antimicrobial factors (2). Pathways for pattern recognition and signaling have been identified in mammals where Toll-like receptors (TLRs)³ (3, 4) and NF-κB provide functions similar to Drosophila Toll and dorsal or Dif, respectively (5, 6).

Innate immunity is induced when loosely defined recognition elements of microbes bind to pattern recognition receptors present on both phagocytic and epithelial cells (7). These receptors include the mannose receptor (8), the GPI-linked LPS co-receptor CD14 (9) and the TLRs (3). In human macrophages, TLR2 acts in concert with CD14 to bind LPS and initiate a signaling cascade (10, 11). The mouse TLR4 homologue is an important determinant of LPS responsiveness (12). In general, activation of pattern recognition receptors induces host defense gene products.

As a primary interface between pathogens and the environment, epithelial cells lining the mammalian airways are a crucial site for the innate immune response. It has been proposed that dysfunction of innate immunity may result in recurrent airway infections as seen in cystic fibrosis (13). The bovine β-defensin tracheal antimicrobial peptide (TAP) serves as a paradigm for induction of innate immunity in the airway (14). The TAP gene is expressed in the ciliated airway epithelium (15) and is induced following experimental bacterial infection (14). In vitro incubation of bovine tracheal epithelial cells with LPS increases TAP mRNA levels via a CD14-mediated response (14), culminating in NF-κB activation and transcriptional up-regulation of the TAP gene (16). Thus, in the bovine airway epithelium antimicrobial peptides are induced through a well defined recognition and activation pathway that helps prevent microbial colonization.

Two β-defensins are present in human epithelia. Human β-defensin-1 (hBD1) (17) is highly expressed in urogenital tissues (18), and to a lesser extent in airway and other epithelia, entirely in a constitutive manner (19). Human β-defensin-2 (hBD2) was initially found in psoriatic skin and is present in cultured keratinocytes in response to bacteria (20). The hBD2 gene is similar to TAP and includes three NF-κB consensus sequences upstream from the transcriptional initiation site (21). The mRNA for hBD2 is present in human lung (22) and is up-regulated in chronic inflammation and by the proinflammatory mediator IL-1β (23). Thus, hBD2 is a host defense molecule whose production is induced in response to infection and inflammation. Purified hBD2 peptide acts synergistically with other antibacterial components of the airway surface fluid, including lysozyme and lactoferrin (22), suggesting a role in maintaining a pathogen-free environment.

As an innate immune response tissue, the human airway must recognize pathogen-associated molecular patterns such as LPS via cell surface receptors. Surprisingly, neither CD14 nor TLRs have been documented in human tracheobronchial epithelial (hTBE) cells. We hypothesized that LPS would trigger hBD2 expression possibly through a CD14-mediated recognition, ultimately resulting in hBD2 induction via activation of NF-κB. To establish this paradigm, we analyzed the hBD2 mRNA response to LPS in hTBE cells and determined the role of CD14. Furthermore, we document expression of TLRs in

* This work was supported by National Institutes of Health Grant HL53400 and Cystic Fibrosis Foundation Grant DIAMONDFO (to G. D.) and Cystic Fibrosis Foundation Grant Randel97Z0 (to S. H. R.).
† To whom correspondence should be addressed: 4006 Thurston-Bowles, CB# 7248, Cystic Fibrosis Center, University of North Carolina, Chapel Hill, NC 27599-7248. E-mail: randell@med.unc.edu.
‡ The abbreviations used are: TLR, Toll-like receptor; hTLR, human Toll-like receptors; LPS, lipopolysaccharide; TAP, tracheal antimicrobial peptide; hBD1 and -2, human β-defensin 1 and 2, respectively; hTBE cell, human tracheobronchial epithelial cell; IL, interleukin; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; GPI, glycosylphosphatidylinositol.

This paper is available on line at http://www.jbc.org 29731
these cells and demonstrate activation of NF-κB in response to LPS.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Escherichia coli LPS serotype O127:B8, Igepal CA-630, nitro blue tetrazolium, and 5-bromo-4-chloro-3-indolylphosphate were from Sigma. Mouse monoclonal My4 and mouse IgG2b antibodies were obtained from Beckman Coulter (Miami, FL). IL-16 was from R&D Systems (Minneapolis, MN).

**Primary Culture of hTBE Cells**—hTBE cells were isolated under the auspices of Institutional Review Board approved protocols as described in detail previously (24). Passage 1 or 2 hTBE cells were cultured at an air-liquid interface on 24-mm T-COL membrane supports (Costar, Cambridge, MA) for the number of days indicated in each experiment. Initial seeding density was 0.7–1 × 10⁵ cells/support. Growth medium was modified from that in Ref. 24 by the use of bovine pituitary extract from Upstate Biotechnology, Inc. (Lake Placid, NY) and the elimination of antibiotics. The medium was periodically tested for endotoxin levels with the Limulus Amebocyte Lysate assay (BioWhittaker (Walkersville, MD) or Associates of Cape Cod (Falmouth, MA)), and endotoxin levels were below 100 pg/ml.

**LPS and Inflammatory Stimulus—LPS (E. coli) at 5 μg/ml was added either apically or basolaterally to cultures with 5% human serum (Sigma catalog no. H4522) as a source of LPS-binding protein.** It is important to note that our cells are normally cultured at an air-liquid interface and that the cells respond to apical flooding by increased acid production (yellowing of the media). In preliminary experiments, we challenged cells with LPS from both sides simultaneously or from only the basal or apical side (with apical flooding controls). The most consistent response was observed with a basal challenge while maintaining an air-liquid interface. Since we observed CD14 on both the apical and basal membrane (this study) and to avoid disturbance of the air-liquid interface, we chose basal challenge for our studies. IL-1β was added basolaterally at 25 ng/ml. For blocking experiments, My4 or IgG2b was added to the cultures both apically and basolaterally 20 min prior to the addition of LPS.

**Northern Blot Analysis**—RNA was isolated with TRI-reagent according to the manufacturer’s protocol (MRC, Cincinnati, OH). Unless otherwise noted, all of the RNA from one 24-mm T-COL membrane was electrophoresed in a single lane on 1.2% agarose/0.1% formaldehyde gels. Ethidium bromide analysis was carried out by standard capillary transfer to a Hybond N membrane (Amersham Pharmacia Biotech). Blots were hybridized with Quikhyb (Stratagene, La Jolla, CA) with 2 × 10⁶ cpm per ml of 32P-labeled probe. For hBD2, either a random primed fragment (bp11–93, accession number U88879) or an end-labeled oligonucleotide (5′-A-CTTGCAGTCCATACGAGGTTACGACCTCAT-3′) was used. Fragments for CD14 (base pairs 52–563, accession number Z71389) or an end-labeled oligonucleotide (5′-A-AGGAGGAGGAAGTGGAGATACGTCAAGTGTTCA-3′) for use as probes. Inserts of hBD2 and CD14 were isolated and random prime-labeled (Rediprime; Amersham Pharmacia Biotech) for use as probes. Inserts for CD14 (base pairs 52–563, accession number Z71389) and γ-actin (Accession number U88540) were annealed and labeled by end filling with Klenow and [32P]dCTP. Binding reactions were performed according to Ref. 29, and complexes were separated by the method of Dignam (27) with the modification that Igepal CA-630 (0.25%) was added to buffer A before homogenization, and buffer D was added directly to the cleared supernatant rather than dialyzed against buffer D. Complementary oligonucleotides containing the NF-κB consensus sequence from the class I MHC promoter (28) were annealed and labeled by end filling with Klenow and [32P]dCTP. Binding reactions were performed according to Ref. 29, and complexes were separated by 5% nondenaturing polyacrylamide gel electrophoresis in Tris-glycine-EDTA buffer. For competition experiments, 20 μg of NF-κB consensus or mutant oligonucleotides from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) were added to the binding reaction.

**RESULTS**

Inducibility of antimicrobial factors by bacterial products and/or inflammatory mediators is a key feature of innate immunity. LPS induction of hBD2 gene expression was examined in passage 1 or 2 hTBE grown in culture at an air-liquid interface. Cells were grown for 7, 14, or 21 days, which corresponds to different stages of mucociliary differentiation (24), and were treated with E. coli LPS at 1 or 5 μg/ml in the presence of human serum as a source of LPS-binding protein. Fig. 1 shows that hBD2 mRNA levels are highest early in culture and that expression is induced after incubation with LPS. In subsequent experiments conducted at 7 days postseeding, steady-state levels of hBD2 mRNA increased an average of 3-fold following LPS (range 1.3–6.3-fold, 10 separate experiments, with seven different patient cell samples) and up to 16-fold following IL-1β stimulation (data not shown), which is consistent with published results (25, 30).

The LPS dose-response relationship for hBD2 induction in hTBE cells is shown in Fig. 2A, which is a graphical representation of the results from a Northern blot with triplicate samples. An LPS dose of 10 ng/ml, which typically induces strong responses in monocyte-derived cells, did not alter hBD2 expression in hTBE cells, but hBD2 mRNA increased following 100 ng/ml to 1 μg/ml of LPS. As shown in Fig. 2B, we examined LPS stimulation of the proinflammatory cytokine IL-8 in the same cultures. The basal expression of IL-8 is approximately 10-fold greater than mRNAs encoding hBD2 and NF-κB.

**TABLE I**

| Gene (accession no.) | Forward primer (5′→3′) | Bases | Reverse primer (5′→3′) | Bases |
|----------------------|------------------------|-------|------------------------|-------|
| γ-Actin (M19283)     | GCAACAGAGAGAAGATGAC    | 1350–1369 | AGGAAAGGACGTTGAGAC   | 2087–2070 |
| TLR1 (U88540)        | TGCCCTGCCTATAGGCA       | 388–398 | GAACACATCCTGACAGCT    | 938–918  |
| TLR2 (U88878)        | TTGCTGATCAAACTGCATCGGC | 2232–2246 | ATCTCCACCAACTGCATCC  | 992–900  |
| TLR3 (U88879)        | CGCCCACTTCAACAGGTA      | 277–294 | GGAAGCAGAAAGAGGAAGA   | 966–949  |
| TLR4 (U88880)        | AGATGGGGCATATCAGAGC     | 446–464 | CCAGAAGCCAAAGATGAGAC  | 945–927  |
| TLR5 (U88881)        | TTCTGACTGATTAAAGGGGC    | 93–113 | TGTGCAAGGAGCTTCTGCA   | 660–641  |
| TLR6 (AB020807)      | CTCACCACTCATGAAAGGAC    | 832–852 | CACCACTATCTCTCAACCCAA   | 1363–1342 |
higher in the presence of serum than in cultures with media alone (data not shown). However, ≥ 1 μg/ml LPS induced a significant increase over this elevated baseline.

The time course of hBD2 mRNA induction is shown in Fig. 3A. LPS increased hBD2 mRNA at 12 and 24 h, but not 6 h, following challenge. Basal hBD2 expression declined over 24 h, possibly in response to the serum added to the medium as a source of LPS-binding protein. Subsequent analyses were performed 24 h following LPS addition. Fig. 3B demonstrates a corresponding LPS-induced increase in hBD2 protein as detected by Western blot.

The GPI-linked cell surface protein, CD14, is known to participate in LPS responsiveness in bovine tracheal epithelial cells (14). We examined hTBE cells for the expression and production of CD14, as well as its involvement in the up-regulation of hBD2. Immunoprecipitation of biotinylated surface proteins reveals that membrane-bound CD14 is found on the apical and basolateral surfaces of hTBE cells (Fig. 4A). The Northern blot shown in Fig. 4B demonstrates that CD14 is also expressed by bovine and murine epithelial cells (14, 38). Our results demonstrate both CD14 mRNA and cell surface protein in human airway epithelial cells. We found that CD14 mRNA was not increased in response to LPS, whereas phagocytic cells or bovine epithelial cells are activated by 10–20 ng/ml concentration of LPS. This data suggest that a peptide-based antimicrobial host response during infection and inflammation is intrinsic to mucosal surfaces.

The molecular pathway for the induction of antimicrobial peptides in the airway is both similar to and different from circulating professional phagocytes, such as monocytes. While initially discovered and extensively studied in myeloid-derived cells, CD14 is also expressed by bovine and murine epithelial cells (14, 38). Our results demonstrate both CD14 mRNA and cell surface protein in human airway epithelial cells. We found that CD14 mRNA was not increased in response to LPS, whereas it is in monocytes (39). The CD14-specific antibody, My4, inhibited LPS-induced hBD2 expression in hTBE cells, which suggests a critical role for CD14 in the mechanism by which airway epithelial cells recognize and respond to bacterial products.

Induction of hBD2 or IL-8 in hTBE requires relatively high concentrations (1–5 μg/ml) of LPS, whereas phagocytic cells or bovine epithelial cells are activated by 10–20 ng/ml concentrations. Thus, while CD14 is an important mediator of LPS responsiveness in hTBE cells, initiation and coupling to downstream signal transduction events appears to be much less efficient than in monocyte-derived cells. Primary cultures of bovine tracheal epithelial cells respond to lower levels of LPS.
Fig. 3. LPS induction of hBD2 mRNA and protein. A, hTBE cells cultured for 7 days were treated with LPS (5 μg/ml) for 6, 12, or 24 h. RNA was isolated, and the resulting Northern blot was hybridized to hBD2 and γ-actin probes. Open boxes are the mean ± S.E. of triplicate values. Although LPS induction of hBD2 was relatively low in this experiment, the 24-h time point was significantly greater than control when compared by Student's t test with Bonferroni's multiple comparison correction. p values for 6, 12, and 24 h were 0.44, 0.08, and 0.01, respectively. B, acid urea-polyacrylamide gel electrophoresis Western blot analysis of hBD2 in acid-soluble proteins from hTBE cells. Lanes 1–3 represent 10, 3, and 1 ng of hBD2 standard. Proteins in lanes 4–7 were isolated from a single 24-mm hTBE culture exposed for 24 h to medium alone, medium plus serum, medium plus serum and LPS, or medium with IL-1 from hTBE cells.

A.U.

Fig. 4. The role of CD14 in the LPS-mediated induction of hBD2 gene expression. A, biotinylated surface proteins were immunoprecipitated with the My4 antibody specific to CD14 or an IgG2b isotype control. Electrophoresed immunoprecipitated proteins were blotted and then visualized by streptavidin-conjugated horseradish peroxidase and chemiluminescent detection. As a positive control, HL60 cells, a myeloid cell line, were differentiated with 50 μM vitamin D. B, My4, a neutralizing antibody to CD14, was added to 7-day-old hTBE cultures at 5 μg/ml both apically and basolaterally for 20 min prior to 24-h basolateral LPS stimulation. IgG2b was used as an isotype control. RNA was isolated from the cultures, and the resulting Northern blot was hybridized sequentially with probes for hBD2, CD14, and γ-actin. The blot shown is representative of three experiments. C, a graphical representation of the blot in B. hBD2 signals were standardized to γ-actin and are presented in arbitrary units. When analyzed by analysis of variance, there was a very significant overall treatment effect (p < 0.001). My4 abolished LPS induction of hBD2 mRNA, (p < 0.001 for LPS alone versus My4 plus LPS according to the Tukey test for multiple comparisons). The antibody control for My4 (IgG2b plus LPS) is significantly greater than its own control (IgG2b alone), p < 0.05. A.U., arbitrary units.

Fig. 5. Expression of Toll-like receptors on hTBE cells. A, RT-PCR analysis of hTLR1–6 with total mRNA from hTBE cells. The absence of genomic DNA was verified by an intron-spanning primer pair from the γ-actin gene. All bands are of the predicted size. RNA isolated from 7-day-old hTBE cultures with and without LPS induction was blotted and hybridized with either hTLR2 (B) or hTLR4 (C) and subsequently with γ-actin probes. (data not shown). As demonstrated by others, LPS desensitization of monocytic cell lines and murine macrophages appears to require 20–100 ng of LPS/ml (40–42). In LPS-tolerant cells, DNA binding activity in NF-κB gel shift assays consists mostly of p50 homodimers in LPS-tolerant cells (40, 41). The NF-κB gel shift assays in our studies are consistent with those seen in LPS-sensitive Mono Mac 6 cells, where the low mobility upper band representing the p50/p65 heterodimer is more prominent. We hypothesize that the low LPS responsiveness of hTBE cells reflects the low level of CD14 expression compared with monocytic cells. In support of this hypothesis are the findings that the response to LPS in THP-1 cells correlates inversely with the level of CD14 expression (43).

The lack of a cytoplasmic domain in GPI-anchored CD14 implies that it acts in concert with other proteins to transduce signals. Several lines of evidence suggest that TLRs contribute...
transmembrane signaling functions. Recent reports indicate that TLR2 mediates both LPS sensitivity (44) and responsiveness to Gram-positive bacteria (45) in transfected 293 cells. However, TLR2 null hamster macrophages still respond to LPS (46), suggesting a complementary function for other TLRs including TLR4. It is now known that mouse TLR4 is equivalent to the mouse LPS gene conferring LPS sensitivity (12). Using RT-PCR, we demonstrate mRNA for the six published hTLR sequences in hTBE cells and have established that hTLR2 and -4 are present at levels detectable by Northern blot of whole RNA. Further studies are needed to clearly elucidate the specific roles of TLR2 and/or -4 in initiating signal transduction responses in the airway. This will require the development of tools including neutralizing antibodies analogous to My4, successful strategies to generate dominant negative phenotypes in difficult to transfect polarized hTBE cells, and genetically engineered animals.

Studies of the Drosophila Toll pathway for antimicrobial factor induction and the discovery of inducible antimicrobial peptide expression in mammals suggest an evolutionarily conserved activation pathway. MUC2 mucin, which can be considered an engineered animals.

Acknowledgments—We gratefully acknowledge Diana Walstad and Ron Kim for cell isolations; Dr. Marty W. Mayo for help with electrophoretic mobility shift assay using Valore and Dr. Alex Cole for advice about acid urea-polyacrylamide gel electrophoresis Western blots; and Dr. Lawrence Ostrowski and Dr. Larry Johnson for invaluable scientific discussions.

REFERENCES

1. Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.-M., and Hoffmann, J. A. (1996) Cell 86, 973–983.
2. Wu, L. P., and Anderson, K. V. (1999) Nature 399, 93–97.
3. Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A., and Bazan, F. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 588–593.
4. Takeuchi, O., Kawai, T., Sanjo, H., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Takeda, K., and Kashiwabara, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 588–593.
5. Medzhitov, R., Preston-Hurlburt, P., Kopp, E., Stadlen, A. Chen, G., Ghosh, S., and Janeway, C. A., Jr. (1998) Mol. Cell. 2, 253–258.
6. Medzhitov, R., Preston-Hurlburt, P., and Janeway, C. A., Jr. (1997) Nature 388, 397–397.
7. Diamond, G., Legarda, D., and Ryan, L. K. (2000) Immunol. Rev. 173, 27–38.
8. Fraser, I. P., Kosiel, H., and Ezekowitz, R. A. B. (1998) Semin. Immunol. 10, 363–372.
9. Ulevitch, R. J., and Tobias, P. S. (1995) Annu. Rev. Immunol. 13, 437–57.
10. Yang, R. B., Mark, M. R., Gurney, A. L., and Godowski, P. J. (1999) J. Immunol. 163, 639–645.
11. Fehrman, E., Wesche, H., Merrill Ayres, T., and Rothe, M. (1998) J. Exp. Med. 188, 2091–2097.
12. Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., and Beutler, B. 1998 Science 282, 2085–2088.
13. Smith, J. J., Travis, S. M., Greenberg, E. B., and Welsh, M. J. (1996) Cell 85, 229–236.
14. Diamond, G. Russell, J. P., and Bevins, C. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5156–5160.
15. Diamond, G., Jones, D. E., and Bevins, C. L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4996–4999.
16. Diamond, G., Kaiser, V., Rhodes, J. Russell, J. P., and Bevins, C. L. (2000) Infect. Immun. 68, 113–119.
17. Benach, K. W., Rada, M., Magert, H.-J., Schulze-Knappe, P., and Forssmann, W.-G. (1995) FEBS Lett. 368, 331–335.
18. Valore, E. V., Park, C. H., Quayle, A. J., Wiles, R. K., McCray, P. B., and Ganz, T. (1998) J. Clin. Invest. 102, 1633–1642.
19. Zhao, C., Wang, I., and Lehrer, R. I. (1996) FEBS Lett. 396, 319–322.
20. Harder, J., Bartels, J. Chistophers, E., and Schroder, J.-M. (1997) Nature 387, 361.
21. Liu, L., Wang, L., Jia, H.-P., Zhao, C., Heng, H. H. Q., Schuttte, B. C., McCray, P. B., and Ganz, T. (1998) Gene (Amst.) 222, 237–244.
22. Bals, R., Wang, X., Wu, Z., Freeman, T., Bafna, V., Zasloff, M., and Wilson, R. C. (1998) J. Virol. 72, 794–803.
23. Singh, P. K., Jia, H. P., Wiles, K., Hesselberth, J., Liu, L., Conway, B. A. D., Welsh, M. J., and Welsh, M. J. (1998) J. Clin. Invest. 102, 874–886.
24. Singh, P. K., Jia, H. P., Wiles, K., Hesselberth, J., Liu, L., Conway, B. A. D., Welsh, M. J., and Welsh, M. J. (1998) J. Clin. Invest. 102, 874–886.
25. Gunning, P. Ponte, P., Okayama, H., Engel, J., Blau, H., and Kedes, L. (1983) Mol. Cell. Biol. 3, 787–795.
26. Pickles, R. J., McCarty, D., Matsui, H., Hart, P. J., Randell, S. H., and Boucher, R. C. (1998) J. Viral. 72, 638–643.
27. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489.
28. Scheimann, R. I., Beg, A. A., and Baldwin, A. S., Jr. (1993) Mol. Cell. Biol. 13, 6089–6101.
29. Ausubel, F. M. (ed) (1994) Current Protocols in Molecular Biology, Vol. 2, 12.0.3–12.2.7, John Wiley & Sons, Inc., New York.
30. Mathews, M., Jia, H. P., Guthmiller, J. M., Losh, G., Graham, S., Johnson, K. G., Tack, B. F., and McCray, P. B., Jr. (1999) Infect. Immun. 67, 2740–2745.
31. O’Neill, J. A., Young, D. W., Welsh, M. J., and Ganz, T. (1998) J. Biol. Chem. 273, 9111–9116.
32. Small, D. S., Nelson, A. L., Abdullah, L., Sheehan, J. K., Harris, A., and Williams Davis, C., and Randell, S. H. (1999) Am. J. Respir. Cell Mol. Biol. 20, 595–604.
33. Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H., and Kedes, L. (1983) Mol. Cell. Biol. 3, 787–795.
34. Pickles, R. J., McCarty, D., Matsui, H., Hart, P. J., Randell, S. H., and Boucher, R. C. (1998) J. Viral. 72, 638–643.
35. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489.
36. Scheimann, R. I., Beg, A. A., and Baldwin, A. S., Jr. (1993) Mol. Cell. Biol. 13, 6089–6101.
37. Ausubel, F. M. (ed) (1994) Current Protocols in Molecular Biology, Vol. 2, 12.0.3–12.2.7, John Wiley & Sons, Inc., New York.
38. Mathews, M., Jia, H. P., Guthmiller, J. M., Losh, G., Graham, S., Johnson, K. G., Tack, B. F., and McCray, P. B., Jr. (1999) Infect. Immun. 67, 2740–2745.
39. O’Neill, J. A., Young, D. W., Welsh, M. J., and Ganz, T. (1998) J. Biol. Chem. 273, 9111–9116.
40. Small, D. S., Nelson, A. L., Abdullah, L., Sheehan, J. K., Harris, A., and Williams Davis, C., and Randell, S. H. (1999) Am. J. Respir. Cell Mol. Biol. 20, 595–604.
41. Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H., and Kedes, L. (1983) Mol. Cell. Biol. 3, 787–795.
Exp. Med. 181, 857–866
39. Marchant, A., Duchow, J., Delville, J. P., and Goldman, M. (1992) Eur. J. Immunol. 22, 1663–1665.
40. Ziegler-Heitbrock, H. W., Wedel, A., Schraut, W., Strobel, M., Wendelgass, P., Sterndorf, T., Bauerle, P. A., Haas, J. G., and Riethmuller, G. (1994) J. Biol. Chem. 269, 17001–17004.
41. Kastenbauer, S., and Ziegler-Heitbrock, H. W. (1999) Infect. Immun. 67, 1553–1559.
42. Nomura, F., Akashi, S., Sakao, Y., Sato, S., Kawai, T., Matsumoto, M., Nakanishi, K., Kimoto, M., Miyake, K., Takeda, K., and Akira, S. (2000) J. Immunol. 164, 3476–3479.
43. Pugin, J., Kravchenko, V. V., Lee, J. D., Klene, L., Ulevitch, R. J., and Tobias, P. S. (1998) Infect. Immun. 66, 1174–1180.
44. Yang, R.-B., Mark, M. R., Gray, A., Huang, A., Xie, M. H., Zhang, M., Goddard, A., Wood, W. I., Gurney, A. L., and Godowski, P. J. (1998) Nature 395, 284–288.
45. Schwandner, R., Dziarski, R., Wesche, H., Rothe, M., and Kirschning, C. J. (1999) J. Biol. Chem. 274, 17406–17409.
46. Heine, H., Kirschning, C. J., Lien, E., Monks, B. G., Rothe, M., and Golenbock, D. T. (1999) J. Immunol. 162, 6971–6975.
47. Li, J.-D., Feng, W., Gallup, M., Kim, J.-H., Gum, J., Kim, Y., and Basbaum, C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5718–5723.
48. Young, R. S., Jones, A. M., and Nicholls, P. J. (1998) J. Pharm. Pharmacol. 50, 11–17.