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CUTTING EDGE

Cutting Edge: TLR4 Deficiency Confers Susceptibility to Lethal Oxidant Lung Injury[1]

Xuchen Zhang, Peiying Shan,* Salman Qureshi,† Robert Homer,‡ Ruslan Medzhitov,§ Paul W. Noble,* and Patty J. Lee[2]**

***TLRs have been studied extensively in pathogen-mediated host responses. We use a murine model of lethal oxidant-mediated injury to demonstrate for the first time that mammalian TLR4 is required for survival and lung integrity. Administering high levels of inspired oxygen, or hyperoxia, is commonly used as a life-sustaining measure in critically ill patients. However, prolonged exposures can lead to respiratory failure and death. TLR4-deficient mice exhibited increased mortality and lung injury during hyperoxia. The enhanced susceptibility of TLR4-deficient mice to hyperoxia was associated with an inability to up-regulate Bcl-2 and phospho-Akt. Restoration of Bcl-2 and phospho-Akt levels by the exogenous transfer of the antioxidant gene heme oxygenase-1 markedly attenuated hyperoxia-induced injury, apoptosis, and mortality in TLR4-deficient mice. Taken together, our results suggest a protective role of TLR4 in oxidant-mediated injury, providing novel mechanistic links among innate immunity, oxidant stress, and apoptosis. The Journal of Immunology,* 2005, 175: 4834–4838.**

Inhalation of high levels of oxygen (hyperoxia) is a necessary and life-saving component in the treatment of critically ill patients. However, prolonged hyperoxia leads to excessive oxidant stress via the accumulation of reactive oxygen species (ROS)[3] that initiate epithelial and endothelial cell death, increased pulmonary capillary permeability, inflammation, lung destruction, and ultimately death (1). ROS have been linked to TLR4 activation and signaling (2). Functional TLR4 has recently been described in the lung (3), but its role in the lung is poorly understood. The lungs, unlike many other organs, are constantly exposed to both microbial agents as well as ambient oxygen and have therefore evolved an extensive system of innate immune and antioxidant defenses, previously thought to be distinct pathways. Our data suggest that TLR4 may mediate a common pathway for innate immune, antioxidant, and antiapoptotic responses.

Generally, TLR4 deficiency is thought to be protective against models of injury such as endotoxin, ischemia-reperfusion, and ozone (4–6). To the best of our knowledge, we demonstrate for the first time that TLR4 is essential for survival in vivo and in lung structural cells during lethal hyperoxic exposure. Furthermore, we use exogenous delivery of heme oxygenase (HO)-1 as both an antioxidant and antiapoptotic strategy to successfully rescue $TLR4^{-/-}$ mice from oxidant-induced death. HO is the rate-limiting enzyme that degrades heme into bilirubin, free iron, and CO (7). Three isoforms of HO exist: HO-2 and -3 are constitutively expressed, whereas HO-1 is the inducible isoform and thought to function as an antioxidant (8). The ability of HO-1 to restore Bcl-2 and phospho-Akt protein expression as well as improve survival in $TLR4^{-/-}$ mice highlights the role of TLR4 in maintaining antioxidant and antiapoptotic balance in the lung.

Materials and Methods

Mice

$TLR4^{-/-}$ (B6;129Tlr4^{tm1Aki}) and the control mice (B6;129F2) have been described previously (9). The $TLR4^{-/-}$ mice were originally provided by S. Akira (Osaka University, Osaka, Japan) (4). Mice were maintained and bred under specific pathogen-free conditions at the animal facility of Yale University School of Medicine. All of the protocols were reviewed and approved by the Animal Care and Use Committee at Yale University.

Murine hyperoxia exposure

Mice were exposed to 100% oxygen (O_2) in a Plexiglas exposure chamber. Naive mice were kept at room air. For survival studies, animals were carefully monitored and the time of death noted. Lung injury was assessed 72 h after the initiation of hyperoxia by performing bronchoalveolar lavage (BAL) cell counts and protein analyses. Lung specimens were also processed for histology, RNA and protein extraction, apoptosis, and immunohistochemistry analyses.

Cell culture and hyperoxia exposures

Lung endothelial cells and type II alveolar epithelial cells were isolated from lungs of wild-type (WT) and $TLR4^{-/-}$ mice with modification of the methods described previously (10). Hyperoxic conditions were achieved as described previously (11).

*Section of Pulmonary and Critical Care Medicine, Yale University School of Medicine, New Haven, CT 06520; †Centre for the Study of Host Resistance, McGill University, Montreal, Quebec, Canada; ‡Department of Pathology, Yale University School of Medicine, New Haven, CT 06520; and §Howard Hughes Medical Institute and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

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[2] Address correspondence and reprint requests Dr. Patty J. Lee, Section of Pulmonary and Critical Care Medicine, Yale University School of Medicine, P.O. Box 208057, New Haven, CT 06520. E-mail address: patty.lee@yale.edu

[3] Abbreviations used in this paper: ROS, reactive oxygen species; HO, heme oxygenase; O_2, oxygen; BAL, bronchoalveolar lavage; WT, wild-type; Ad-HO-1, adenoviral HO-1; Ad-LacZ, adenoviral β-galactosidase.

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Apoptosis assays

TUNEL assay was performed, and TUNEL-positive cells were expressed as a percentage to total cells as described previously (11). Flow cytometry was performed on cells using a FACS to detect Annexin V-FITC labeling (BD Pharmingen) as described previously (12).

Western blot analysis

Protein levels of Bcl-2, Bad, Bax, caspase-3 (Santa Cruz Biotechnology), HO-1 (StressGen Biotechnologies), and phospho-Akt (Cell Signaling Technology) were analyzed by Western blot assays as described previously (13).

Intranasal administration of recombinant adenovirus-containing HO-1 cDNA

Mice were anesthetized with methoxyflurane, and then 5×10^8 PFU of adenoviral HO-1 (Ad-HO-1) (a gift from L. E. Otterbein, Harvard University, Boston, MA) or adenoviral β-galactosidase (Ad-LacZ) (BD Biosciences) were administered intranasally to each mouse in a volume of 50 μl as described previously (12).

Total RNA isolation and RT-PCR amplification

Total RNA from lung tissue was extracted as described previously (12). For mouse TLR4, sense: GCTTTCACCTCTGCCTTCAC; antisense: CGAG GCTTTTCCATCCAATA; and for mouse β-actin, sense: GTGGGC CGCTCTAGGCACCAA; antisense: CTCTTTGATGTCACGCAC GATTTC. RT-PCR was performed using RT-PCR Master Mix (USB).

Statistics

Data are expressed as mean ± SE and analyzed by Student's t test. Survival studies were evaluated using log-rank analysis (14), and statistical analysis was performed using GraphPad Prism 3.0 (GraphPad) software. Significant difference was accepted at $p < 0.05$.

Results and Discussion

TLR4 is essential for survival during hyperoxia

To investigate whether TLR4 and its endogenous ligands are involved in hyperoxia-induced lung injury, we exposed WT mice to hyperoxia for 72 h, a time point previously determined to be most representative of maximal stress responses in the murine lung (15). We found that hyperoxia increased TLR4 mRNA expression in whole lung lysates (Fig. 1*A*). We also confirmed increased TLR4 protein expression by immunohistochemistry in a variety of lung cells (including lung epithelial and endothelial cells) after hyperoxia (data not shown). Hyperoxia also increased expression of the endogenous TLR4 ligands fibronectin and hyaluronan in both WT and TLR4-deficient mice ($TLR4^{-/-}$) (data not shown). To determine whether there was a functional role in vivo for TLR4 induction during hyperoxia, we assessed survival rates of WT and $TLR4^{-/-}$ mice in continuous hyperoxia. Given that TLR4 deficiency has been shown to be protective in other models of noninfectious injury (4–6), we expected $TLR4^{-/-}$ mice would be less susceptible to the damaging effects of hyperoxia. To our surprise, $TLR4^{-/-}$ mice showed significantly increased mortality during hyperoxia compared with WT mice (Fig. 1*B*). After 5 days of hyperoxia exposure, 55.6% of the WT mice ($n = 18$) remained alive, whereas none of the $TLR4^{-/-}$ mice ($n = 18$) were alive. The range of survival for $TLR4^{-/-}$ mice was 2.5–5 days, and the range for WT mice was 3.5–6.5 days. This indicated that a TLR4-dependent signaling pathway was critical for survival during hyperoxia. Consistent with the survival data, $TLR4^{-/-}$ mice exposed to hyperoxia exhibited significantly greater inflammation, lung permeability, and oxidative DNA damage compared with WT mice (Fig. 1, *C–E*). Of note, naive $TLR4^{-/-}$ mice and naive WT mice have similar basal levels of BAL cell counts, protein, and DNA oxidation (data not shown).

TLR4 is essential for appropriate antiapoptotic responses during hyperoxia in vivo and in vitro

Both animal and recent patient studies have established apoptosis, specifically lung endothelial and epithelial apoptosis, as an important part of the pathogenesis of acute lung injury (16, 17). We found that $TLR4^{-/-}$ mice exhibited significantly greater lung TUNEL staining (Fig. 2*A*). Both epithelial and endothelial cells isolated from $TLR4^{-/-}$ mice also showed significantly more apoptosis than cells from WT mice during hyperoxia (Fig. 2, *B* and *C*).

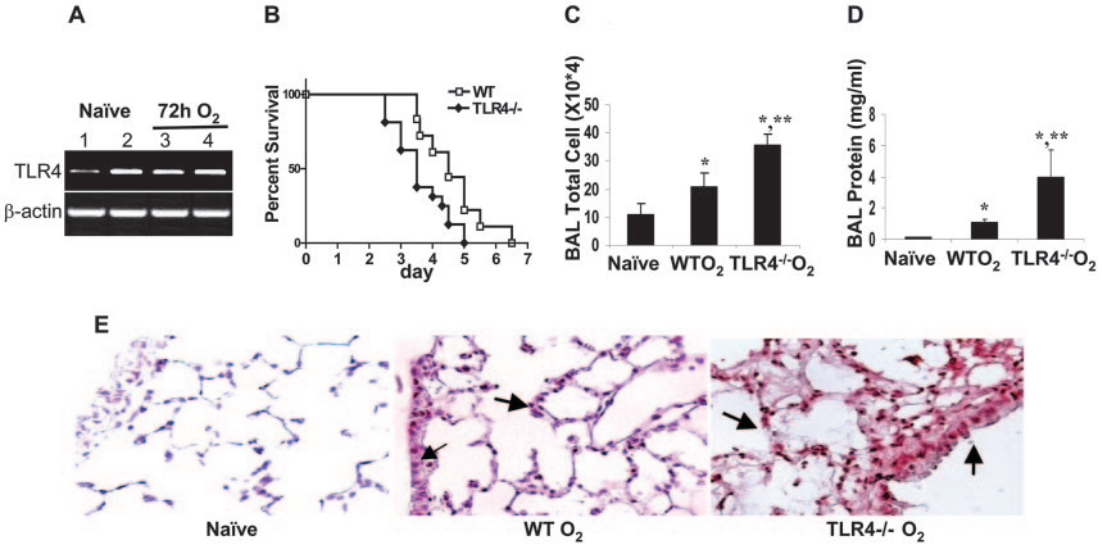

FIGURE 1. TLR4 is essential for survival during hyperoxia. *A*, RT-PCR analysis for TLR4 mRNA expression in lung lysates from WT naive mice (*lane 1*) and mice exposed to 72-h O_2 (*lanes 2–4*). *B*, Survival curves of WT ($n = 18$) and $TLR4^{-/-}$ ($n = 18$) mice exposed to hyperoxia ($p = 0.004$, WT vs $TLR4^{-/-}$). *C*, Lung inflammation was detected by BAL cell counts in naive mice, WT mice, and $TLR4^{-/-}$ mice exposed to 72-h O_2. *D*, Lung permeability was assessed by BAL protein. Data represent mean ± SE. *, $p < 0.05$ compared with naive mice; **, $p < 0.05$ compared with WT O_2. *E*, DNA oxidation was detected by 8-hydroxy-2′-deoxyguanosine immunohistochemical staining in the lungs of naive, WT mice exposed to 72-h O_2, and $TLR4^{-/-}$ mice exposed to 72-h O_2. Arrows indicate positive red staining (×400, original magnification). Results shown are representative of three to five independent experiments.

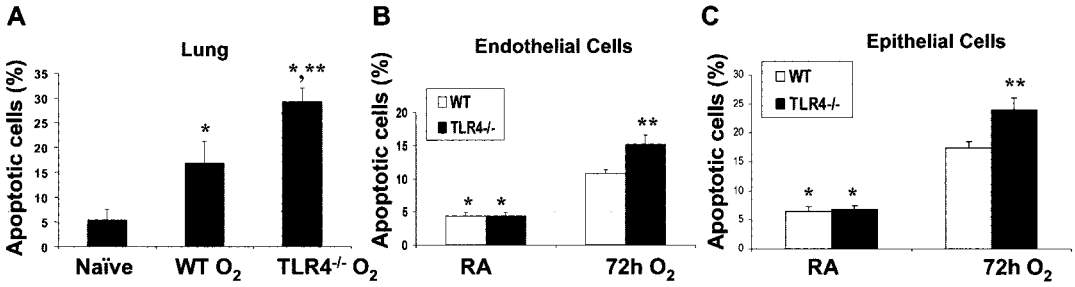

FIGURE 2. TLR4 is essential for lung endothelial and epithelial survival during hyperoxia. *A*, Lung sections were processed for TUNEL staining and quantitation expressed as percentage of total cells. Data are shown as mean ± SE. *, $p < 0.05$ compared with naive mice; **, $p < 0.05$ compared with WT 72-h O_2. *B*, Apoptosis of lung endothelial cells isolated from WT and TLR4$^{-/-}$ mice was measured by flow cytometry after 72-h O_2. *C*, Apoptosis of lung alveolar type II cells isolated from WT and TLR4$^{-/-}$ mice was measured by flow cytometry after 72-h O_2. Graphical representation of the data is shown as mean ± SE. *, $p < 0.05$ compared with corresponding 72-h O_2; **, $p < 0.05$ compared with WT 72-h O_2. Results shown are representative of three to five independent experiments.

We postulated that a potential mechanism of increased lung apoptosis in TLR4$^{-/-}$ mice might be differences in anti- and proapoptotic protein expression. Our Western blot results showed that hyperoxia increased expression of Bcl-2 in WT lungs (Fig. 3). However, TLR4$^{-/-}$ mice were unable to significantly increase Bcl-2 protein in their lungs. The expression of Bad and Bax appeared relatively unchanged during hyperoxia in the WT and TLR4$^{-/-}$ mice. The Akt pathway is also known to play an important role in modulating apoptosis and was recently linked to TLR4 in response to LPS (18, 19). We found that TLR4$^{-/-}$ mice were unable to increase phospho-Akt expression in response to hyperoxia, unlike WT mice (Fig. 3). In addition, TLR4$^{-/-}$ mice have exaggerated levels of activated caspase-3 expression in lungs, as assessed by Western blot analysis, during hyperoxia compared with WT mice (Fig. 3). These data indicated that the mechanism of increased lung apoptosis and injury in TLR4$^{-/-}$ mice was the lack of key antiapoptotic responses, namely Bcl-2 and phospho-Akt induction, and increased proapoptotic processes such as caspase-3 activation. TLR4 signaling is generally thought to be proapoptotic, but this has previously been studied in the context of LPS, microbes, or immune cells (20–22). We show both in vivo and in epithelial and endothelial cells isolated from TLR4$^{-/-}$ mice that TLR4 is necessary to maintain appropriate antiapoptotic responses during hyperoxia.

Hyperoxia likely induces lung TLR4 signaling via increased endogenous ligands such as fibronectin and hyaluronan in vivo. Alternatively, ROS may directly "ligate" TLR4 by affecting redox-sensitive moieties of the receptor given that hyperoxia can also induce TLR4 in isolated lung cell culture systems (data not shown). Others have demonstrated the involvement of the endothelial and epithelial cell in TLR signaling (23, 24). Potential explanations as to why TLR4 deficiency is protective in certain settings yet not in others include the use of different mouse strains and injury models. It is clear that distinct TLR signaling pathways are used in response to different types of injury. For instance, unlike LPS or ischemia-reperfusion-induced TLR signaling in which NF-κB mediates TLR signal transduction (25), NF-κB does not appear to have a major role in hyperoxia-induced injury (26).

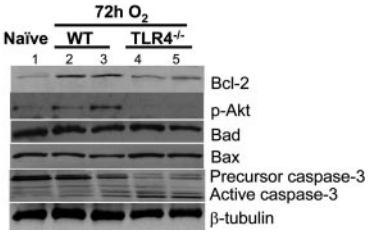

FIGURE 3. TLR4 modulates antiapoptotic protein and caspase-3 expression during hyperoxia. Western blot analysis for pro- and antiapoptotic protein expression in lung lysates from single animals: naive mice (*lane 1*), WT mice exposed to 72-h O_2 (*lanes 2* and *3*), and TLR4$^{-/-}$ mice exposed to 72-h O_2 (*lanes 4* and *5*). Ab to β-tubulin was used as loading control. Results shown are representative of three to five independent experiments.

An antioxidant and antiapoptotic strategy with HO-1 overexpression rescues TLR4$^{-/-}$ mice from lethal hyperoxia

HO-1 and its reaction products exert potent antioxidant and antiapoptotic properties in a variety of injury models (12, 27). We have demonstrated that HO-1 and its reaction product CO have the ability to induce the antiapoptotic proteins Bcl-2 and phospho-Akt (13, 28), both of which are lacking in the TLR4$^{-/-}$ mice. Of note, TLR4$^{-/-}$ mice have the ability to up-regulate HO-1 expression during hyperoxia. However, stress-induced HO-1 expression is likely a consequence of severe oxidant injury, and HO-1 levels as well as timing of induction in this context are inadequate to provide significant protection (12). High levels of HO-1 and its products are required before or at the onset of injury to have beneficial effects. Therefore, we administered intranasal rat HO-1 in an adenoviral vector (Ad-HO-1) to achieve HO-1 overexpression in mouse lungs before hyperoxia exposure. WT mice given Ad-HO-1 showed significantly increased survival during hyperoxia compared with WT mice given empty vector (Ad-LacZ) (Fig. 4*A*). Furthermore, TLR4$^{-/-}$ mice given Ad-HO-1 also showed markedly increased survival compared with TLR4$^{-/-}$ mice given Ad-LacZ, reaching a survival rate comparable to WT/Ad-LacZ mice (Fig. 4*A*). After 5 days of hyperoxia exposure, 50% of TLR4$^{-/-}$ mice with Ad-HO-1 remained alive, whereas all of the TLR4$^{-/-}$ mice with Ad-LacZ were dead. The range of survival for TLR4$^{-/-}$ mice with Ad-HO-1 was 4–6 days, and for TLR4$^{-/-}$ mice with Ad-LacZ the range of survival was 3.5–5 days. The improved survival with Ad-HO-1 correlated with attenuation of lung inflammation, permeability, and oxidative DNA damage in TLR4$^{-/-}$ mice during hyperoxia (data not shown).

In addition, Ad-HO-1 rescued TLR4$^{-/-}$ mice from hyperoxia-induced lung apoptosis (Fig. 4*B*). As expected, Ad-HO-1 led to appropriately increased levels of lung HO-1 protein expression in TLR4$^{-/-}$ compared with TLR4$^{-/-}$ mice given Ad-LacZ before hyperoxia (Fig. 4*C*). Interestingly, Ad-HO-1 restored levels of Bcl-2 and phospho-Akt protein expression in TLR4$^{-/-}$ mice to that of WT mice when challenged with hyperoxia (Fig. 4*C*). This

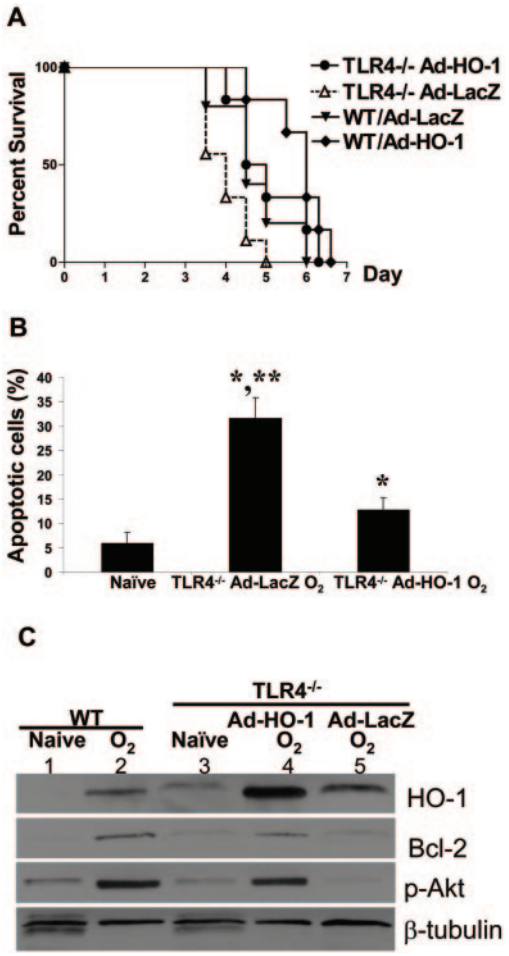

FIGURE 4. Ad-HO-1 gene transfer rescues TLR4$^{-/-}$ mice from hyperoxia-induced mortality and apoptosis. TLR4$^{-/-}$ mice were intranasally administered Ad-LacZ or rat Ad-HO-1 48 h before hyperoxia and then assessed for survival and lung injury. *A*, Survival curves for WT and TLR4$^{-/-}$ administered Ad-HO-1 or Ad-LacZ before hyperoxia ($p = 0.03$, WT/Ad-LacZ vs WT/Ad-HO-1; $p = 0.02$, TLR4$^{-/-}$/Ad-LacZ vs TLR4$^{-/-}$/Ad-HO-1). *B*, Lung sections were processed for TUNEL staining, and quantitation was expressed as percentage of total cells. Data are shown as mean ± SE. *, $p < 0.05$ compared with naive mice; **, $p < 0.05$ compared with TLR4$^{-/-}$ Ad-HO-1 72-h O_2. *C*, Western blot analysis in lung lysates from naive WT (*lane 1*), WT mice exposed to 72-h O_2 (*lane 2*), naive TLR4$^{-/-}$ mice (*lane 3*), TLR4$^{-/-}$ mice administered Ad-HO-1 before 72-h O_2 (*lane 4*), and TLR4$^{-/-}$ mice administered Ad-LacZ before 72-h O_2 (*lane 5*). Results shown are representative of three to five independent experiments.

indicated that TLR4$^{-/-}$ mice retain the ability to induce cytoprotective pathways. Taken together, these data demonstrate that TLR4 is essential for survival, possibly by maintaining appropriate levels of antiapoptotic responses such as Bcl-2 and phospho-Akt induction in the face of oxidant stress. Although links between TLR4 and Akt have been described (19), the precise pathway(s) whereby TLR4 modulates Akt and Bcl-2 during oxidant injury is unknown and will be an important focus of future studies. There is also a possibility that TLR4$^{-/-}$ mice are deficient in multiple signaling pathways, which thus opens up new avenues of investigation.

Our current data point to a novel paradigm for TLR4 signaling in response to exogenous oxidants. TLR4, at least in the lung, appear to be not only important sensors of conserved microbial components but also exogenous oxidants and thereby modulators of downstream responses such as inflammation and apoptosis. This would make teleological sense for an organ that is the first-line defense against inhaled microbial Ags as well as numerous oxidative stressors in the environment. In addition, the mechanisms of TLR4 responses in the lung during oxidant stress are likely to be distinct from what has thus far been described in other systems. Ultimately, these studies bring forth a fundamental link between innate immunity pathways and exogenous, noninfectious injury signals. These new insights will allow broader approaches for the prevention and treatment of a variety of oxidant-mediated disease processes.

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Disclosures

The authors have no financial conflict of interest.

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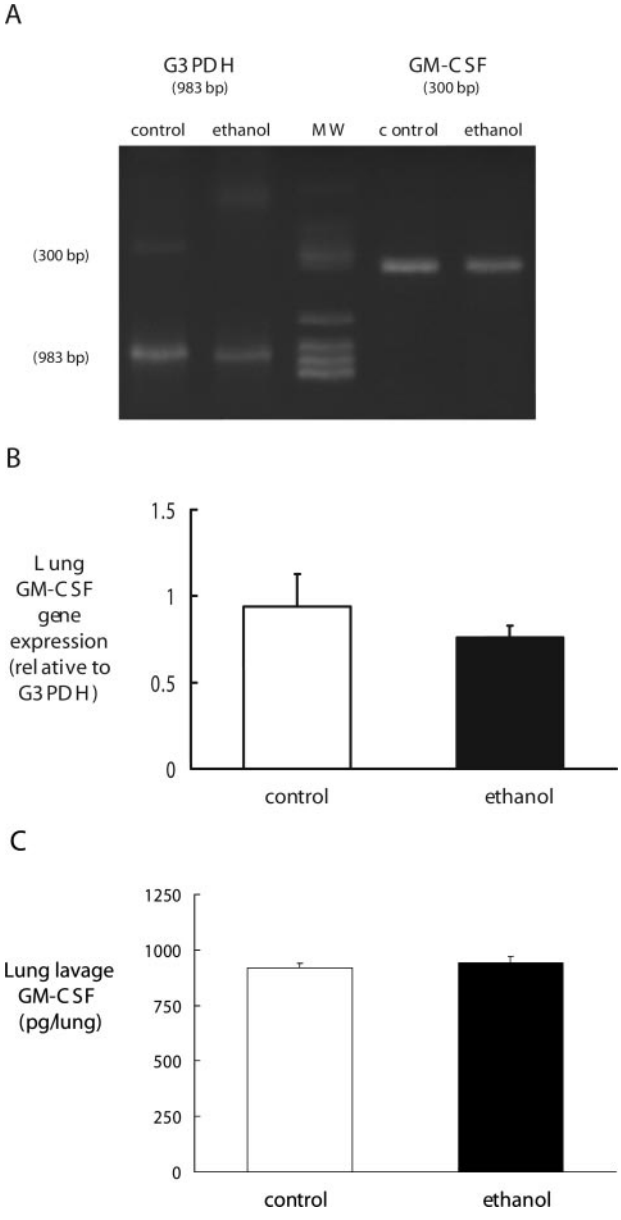
CORRECTIONS

Joshi, P. C., L. Applewhite, J. D. Ritzenthaler, J. Roman, A. L. Fernandez, D. C. Eaton, L. A. S. Brown, and D. M. Guidot. 2005. Chronic ethanol ingestion in rats decreases granulocyte-macrophage colony-stimulating factor receptor expression and downstream signaling in the alveolar macrophage. *J. Immunol.* 175: 6837–6845.

In Figure 1, panel C was omitted. The corrected figure is shown below. The error has been corrected in the online version, which now differs from the print version as originally published.



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In the author line, the sequence of the first two authors is reversed. The corrected author line is shown below.

Krishnamurthy Malathi, Xiaogui Li, Olga Krizanova, Karol Ondrias, Kirk Sperber, Vitaly Ablamunits, and Thottala Jayaraman

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Pasquetto, V., H.-H. Bui, R. Giannino, F. Mirza, J. Sidney, C. Oseroff, D. C. Tscharke, K. Irvine, J. R. Bennink, B. Peters, S. Southwood, V. Cerundolo, H. Grey, J. W. Yewdell, and A. Sette. 2005. HLA-A*0201, HLA-A*1101, and HLA-B*0702 transgenic mice recognize numerous poxvirus determinants from a wide variety of viral gene products. *J. Immunol.* 175: 5504–5515.

The fourth author's name, Cindy Banh, was omitted. The correct list of authors and affiliations is shown below.

Valerie Pasquetto,* Huynh-Hoa Bui,* Rielle Giannino,* Cindy Banh,* Fareed Mirza,[†] John Sidney,* Carla Oseroff,* David C. Tscharke,[§¶] Kari Irvine,[§] Jack R. Bennink,[§] Bjoern Peters,* Scott Southwood,[‡] Vincenzo Cerundolo,[†] Howard Grey,* Jonathan W. Yewdell,[§] and Alessandro Sette[2*]

*La Jolla Institute for Allergy and Immunology, San Diego, CA 92109; [†]Tumor Immunology Unit, Weatherall Institute of Molecular Medicine, Oxford University, Oxford, United Kingdom; [‡]Epimmune Incorporated, San Diego, CA 92121; [§]Laboratory of Viral Diseases, National Institutes of Health, Bethesda, MD 20892; and [¶]Division of Immunology and Infectious Diseases, Queensland Institute of Medical Research, Herston, Queensland, Australia

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Zhang, X., P. Shan, S. Qureshi, R. Homer, R. Medzhitov, P. W. Noble, and P. J. Lee. 2005. Cutting edge: TLR4 deficiency confers susceptibility to lethal oxidant lung injury. *J. Immunol.* 175: 4834–4838.

In **Materials and Methods**, in the first sentence under the heading *Intranasal administration of recombinant adenovirus-containing HO-1 cDNA*, the source for adenoviral HO-1 cDNA was incorrectly attributed. The source is stated in the corrected sentence below.

Mice were anesthetized with methoxyflurane, and then 5×10^8 PFU of adenoviral HO-1 (Ad-HO-1) (a gift from K. Kolls, University of Pittsburgh Medical Center, Pittsburgh, PA, and J. Alam, Alton Ochsner Medical Foundation, New Orleans, LA) (29) or adenoviral β-galactosidase (Ad-LacZ) (BD Biosciences) were administered intranasally to each mouse in a volume of 50 μl as described previously (12).

The authors also wish to add the reference shown below.

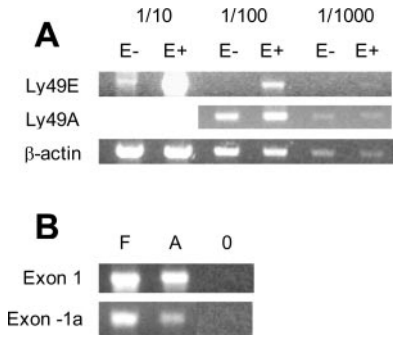
29. Otterbein, L. E., J. K. Kolls, L. L. Mantell, J. L. Cook, J. Alam, and A. M. K. Choi. 1999. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. *J. Clin. Invest.* 103: 1047–1054.

Gays, F., K. Martin, R. Kenefeck, J. G. Aust, and C. G. Brooks. 2005. Multiple cytokines regulate the NK gene complex-encoded receptor repertoire of mature NK cells and T cells. *J. Immunol.* 175: 2938–2947.

In Figure 1, a sentence regarding the solid and broken lines was omitted from the legend. The corrected legend is shown below.

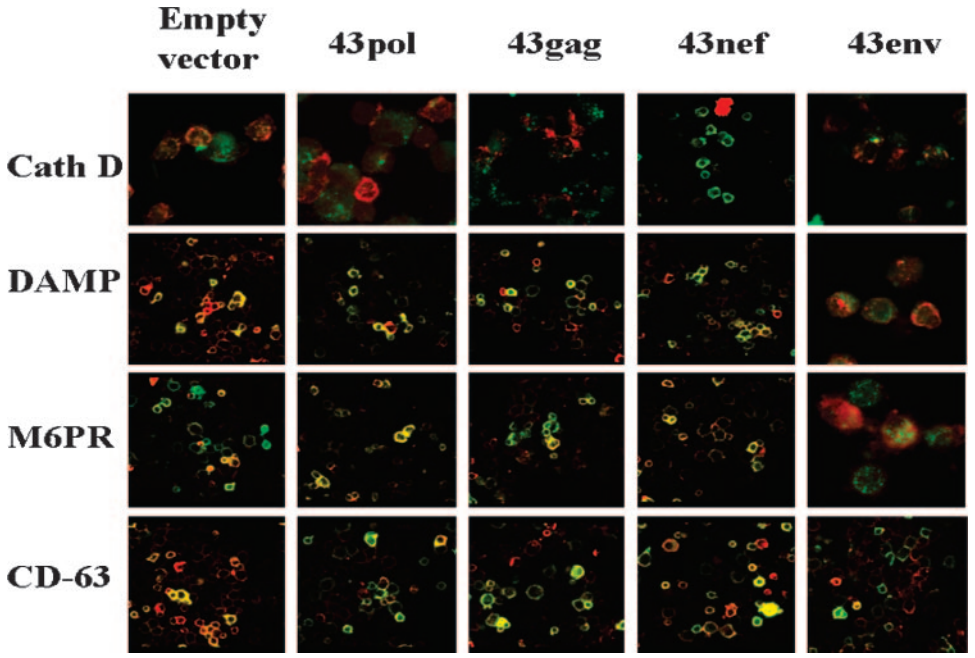
FIGURE 1. Specificity of the CM4 mAb. *A*, YB2 or RNK cells transfected with Ly49 constructs were stained with medium or first layer Abs followed by AF488 goat anti-mouse Ig. Solid lines: staining by CM4. Left broken line: medium control. Right broken line: staining by positive control Abs Ly49A = A1, Ly49B = 1A1, Ly49C = 4D12, Ly49D = 4E5, Ly49E = 4D12, Ly49F = HBF, Ly49G = 4G11, Ly49H = 3D10, Ly49I = YBI. *B*, Cross-competition between Abs. YB2 cells transfected with Ly49E (YB2-E) and RNK cells transfected with Ly49F (RNK-F) were incubated with medium or saturating quantities of the unlabeled Ly49 Abs shown on the y-axis. After 20 min, AF488-labeled CM4, 4D12, or HBF Ab was added, and incubation was continued for an additional 20 min. Median fluorescence values were determined by flow cytometry, and the percentage inhbition caused by pretreatment with each unlabeled Ab is plotted on the y-axis. The likelihood that the inhibition observed was due to chance variation was determined by Student's *t* test (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$). The experiments shown are representative of three similar experiments of each type that were performed.

In Figure 9A, the gel image labeled Ly49A is inverted. The corrected figure is shown below.



Rakoff-Nahoum, S., H. Chen, T. Kraus, I. George, E. Oei, M. Tyorkin, E. Salik, P. Beuria, and K. Sperber. 2001. Regulation of class II expression in monocytic cells after HIV-1 infection. *J. Immunol.* 167: 2331–2342.

Figure 10, demonstrating intracellular trafficking of HLA-DR after the introduciton of HIV proteins, is incorrect. The corrected figure is shown below.



Lukacs, N. W., K. K. Tekkanat, A. Berlin, C. M. Hogaboam, A. Miller, H. Evanoff, P. Lincoln, and H. Maassab. 2001. Respiratory syncytial virus predisposes mice to augmented allergic airway responses via IL-13-mediated mechanisms. *J. Immunol.* 167: 1060–1065.

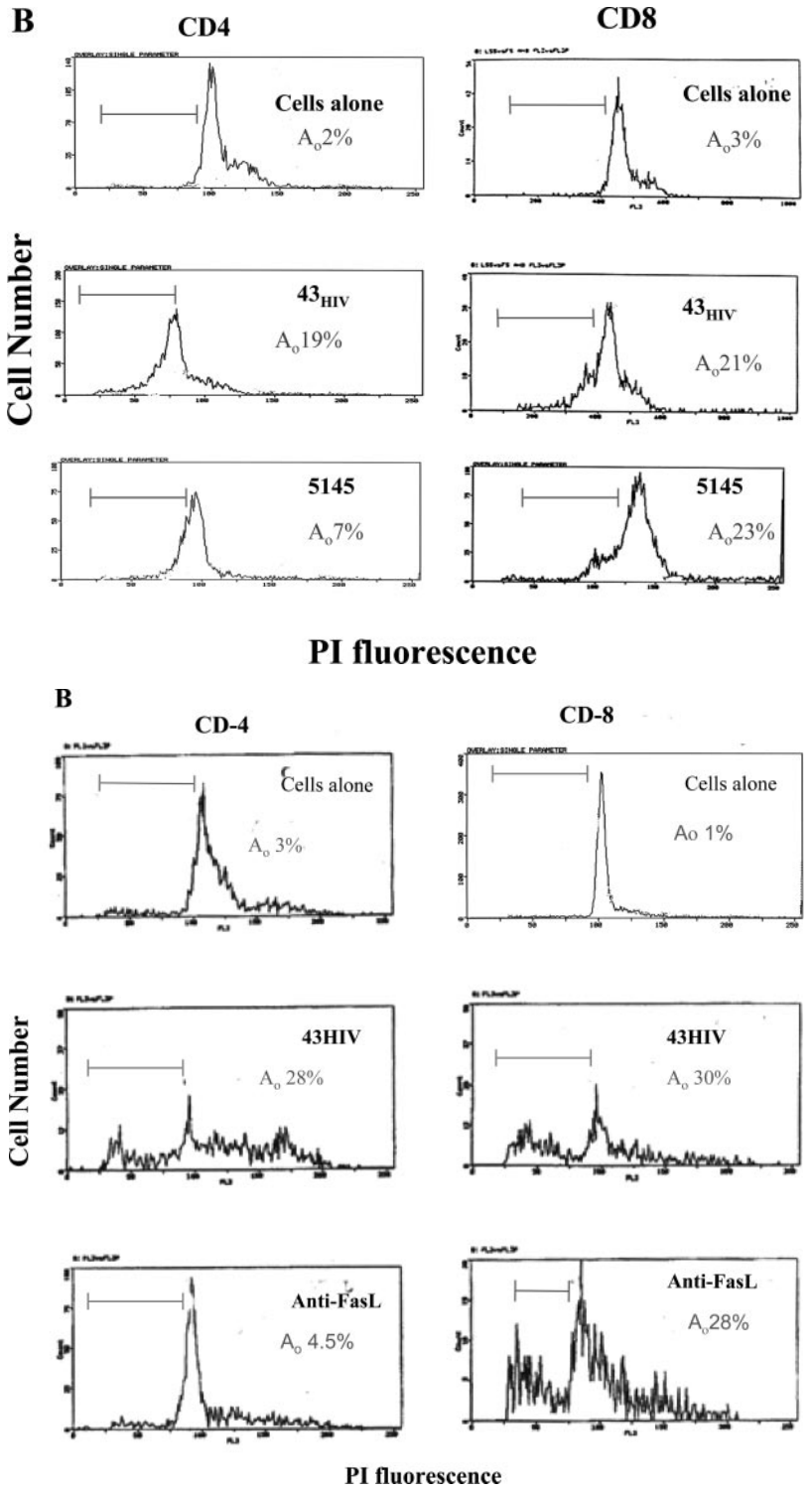
In **Materials and Methods**, in the first sentence under the heading *RSV infection*, the designation of the virus type should be human RSV A strain, not A2 strain.

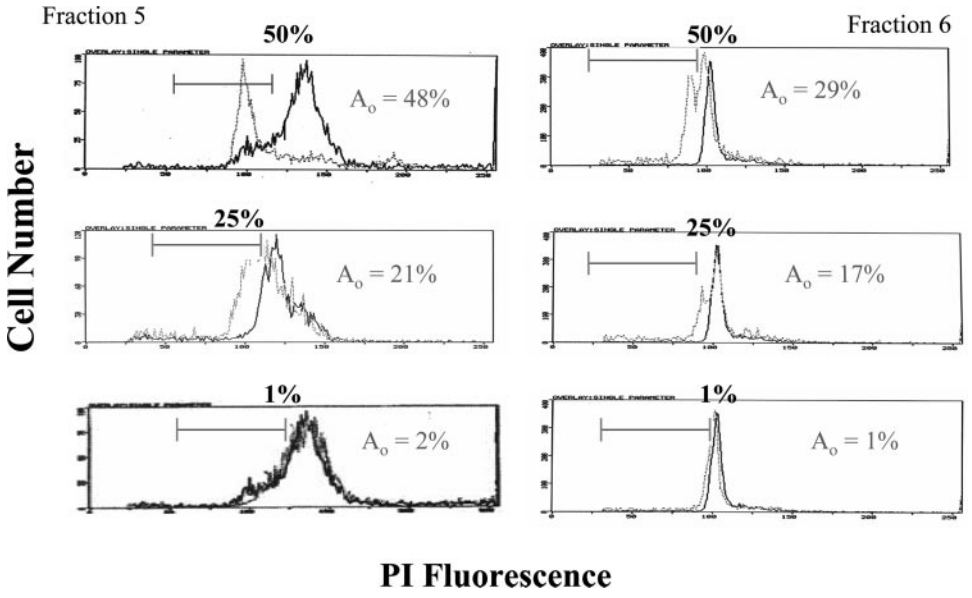
Tekkanat, K. K., H. F. Maassab, D. S. Cho, J. J. Lai, A. John, A. Berlin, M. H. Kaplan, and N. W. Lukacs. 2001. IL-13-induced airway hyperreactivity during respiratory syncytial virus infection is STAT6 dependent. *J. Immunol.* 166: 3542–3548.

In **Materials and Methods**, in the first sentence under the heading *Virus and infection*, the designation of the virus type should be human RSV A strain, not A2 strain.

Chen, H., Y. K. Yip, I. George, M. Tyorkin, E. Salik, and K. Sperber. 1998. Chronically HIV-1-infected monocytic cells induce apoptosis in cocultured T cells. *J. Immunol.* 161: 4257–4267.

Figure 3B, demonstrating the apoptotic effect of gp120 on CD4 and CD8 cells; Figure 4B, depicting the apoptotic effect of Fas-FasL interactions in CD4 and CD8 T cells cocultured with 43_{HIV} cells; and Figure 6B, showing the apoptotic activity of fractionated supernatant from the 43_{HIV} cell line, are inaccurate. The corrected figures are shown below.





Polyak, S., H. Chen, D. Hirsch, I. George, R. Hershberg, and K. Sperber. 1997. Impaired class II expression and antigen uptake in monocytic cells after HIV-1 infection. *J. Immunol.* 159: 2177–2188.

In Figure 5, demonstrating the inability of HIV-1-infected 43 cells to present antigen to HLA-DR2 and DR4 T cells, panels A and B are the same. The corrected figure is shown below.