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NOTES

Role of Hypercytokinemia in NF-κB p50-Deficient Mice after H5N1 Influenza A Virus Infection▽

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During H5N1 influenza virus infection, proinflammatory cytokines are markedly elevated in the lungs of infected hosts. The significance of this dysregulated cytokine response in H5N1-mediated pathogenesis remains to be determined. To investigate the influence of hypercytokinemia, or "cytokine storm," a transgenic mouse technology was used. The classical NF-κB pathway regulates the induction of most proinflammatory cytokines. Deletion of the p50 subunit leads to a markedly reduced expression of the NF-κB-regulated cytokines and chemokines. Here we show that H5N1 influenza virus infection of this transgenic mouse model resulted in a lack of hypercytokinemia but not in altered pathogenesis.

Infections with H5N1 influenza A viruses cause severe illness and high mortality rates in mammalian hosts (15, 21, 23, 30). It has been described previously that this critical disease outcome is associated with a dysregulation of the immune system involving elevated levels of proinflammatory cytokines (cytokine storm or hypercytokinemia). This observation is based on a large variety of studies demonstrating a marked increase of cytokines and chemokines in the lungs of humans and animals during H5N1 influenza A virus infection (2, 8, 12, 19). In addition, the combination of acute respiratory distress syndrome, lymphopenia, and multiorgan failure characteristic of H5N1 disease in humans has previously been associated with cytokine dysregulation (6). It has been demonstrated in vitro that H5N1 infection causes significantly greater expression of cytokines than that induced by seasonal H1N1 or H3N2 infections (5). Nevertheless, hypercytokinemia was also found in macaques infected with the H1N1 influenza A virus responsible for the "Spanish flu" pandemic (11). In contrast, recent studies gave rise to a more controversial view on the role of hypercytokinemia in H5N1-mediated pathogenesis. Studies using transgenic mice lacking one cytokine revealed no altered pathogenesis after lethal H5N1 influenza virus infection (22, 25). However, hypercytokinemia is a multicytokine event (9, 24), so the effect of deleting only one cytokine might be of limited significance. The possibility to interfere with the majority of cytokines and chemokines is provided by the manipulation of the nuclear factor kappa B (NF-κB) pathway. The transcription factor NF-κB plays a central role in the regulation of a large variety of cellular events. It functions as a regulator of the expression of inflammatory cytokines, chemokines, immunoreceptors, and adhesion molecules. There are two different NF-κB pathways (4). The expression of many cytokines and chemokines involved in hypercytokinemia is regulated through the classical NF-κB pathway, which operates through p50 and p65 subunits (18). Thus, using NF-κB p50 knockout mice for H5N1 infection represents a useful model to study the influence of hypercytokinemia in the pathogenesis after H5N1 infection.

To examine the local cytokine production in the lung after H5N1 influenza virus infection of C57BL/6 wild-type (NF-κB $p50^{+/+}$), NF-κB $p50^{+/-}$, and NF-κB $p50^{-/-}$ mice, multiple cytokine assays were performed at 6 days post-H5N1 infection. In our studies, we used a natural H5N1 field isolate, MB1 (A/mallard/Bavaria/1/2006), that is highly pathogenic to mice without adaptation. An upregulation of IP-10, gamma interferon (IFN-γ), interleukin-6 (IL-6), MIG, macrophage inflammatory protein 1β (MIP-1β), RANTES, IFN-β, tumor necrosis factor alpha (TNF-α), IL-2, and IL-10, but not IL-4, was found in the lungs of infected wild-type mice (Fig. 1A to L). These elevated mRNA levels correlated with protein expression of the different chemokines and cytokines in the lung (data not shown). NF-κB $p50^{+/-}$ (Fig. 1, gray bars) and NF-κB $p50^{-/-}$ (Fig. 1, white bars) mice revealed a strong reduction of most cytokines and chemokines after H5N1 infection. The amounts of IL-2, RANTES, MIG, and IP-10 were more reduced in NF-κB $p50^{+/-}$ mice than in NF-κB $p50^{-/-}$ mice (Fig. 1), without an influence on the pathogenesis. The levels of MIP-1β (Fig. 1K) and IP-10 (Fig. 1L) were equal to or even higher than those in wild-type controls. One might argue that the high levels of IP-10 and MIP-1 in wild-type and NF-κB p50-deficient mice are responsible for the severity of disease or pathogenesis. It was previously demonstrated that MIP-1-deficient mice showed no significant difference in mortality compared to wild-type animals after H5N1 infection (25). Experiments with mice deficient in the IP-10 receptor ($CXCR3^{-/-}$ mice) demonstrated identical virus replication and mortality rates after H1N1 infection (27). Taken together, these results indicate that no hypercytokinemia was

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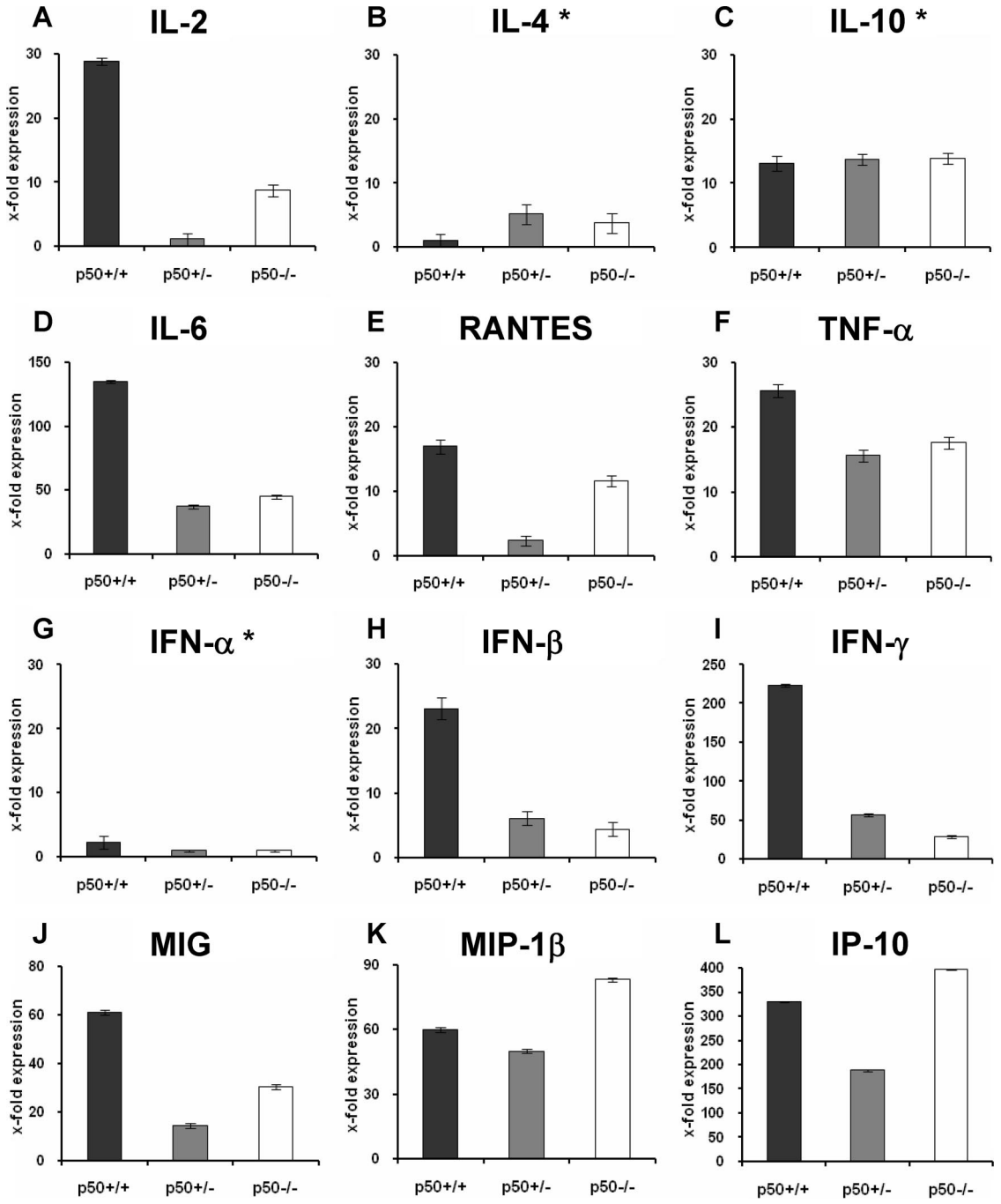

FIG. 1. mRNA levels of cytokines and chemokines at 6 days p.i. with 5×10^3 PFU H5N1 in NF-κB $p50^{+/+}$ (black bars), NF-κB $p50^{+/-}$ (gray bars), and NF-κB $p50^{-/-}$ (white bars) mice after reverse transcriptase real-time PCR performed according to the manufacturer's protocol (Qiagen). (A) IL-2; (B) IL-4; (C) IL-10; (D) IL-6; (E) RANTES; (F) TNF-α; (G) IFN-α; (H) IFN-β; (I) IFN-γ; (J) MIG; (K) MIP-1β; (L) IP-10. The bars represent the mRNA levels for three infected mice compared to those for uninfected controls according to Boeuf et al. (3). This experiment was performed twice with similar results. *, Student's *t* test revealed no significant difference between the three NF-κB mouse strains ($P > 0.1$). The amounts of cytokines were also analyzed at the protein level by use of Bio-Plex arrays, except for MIG, IP-10, and IFN-β, revealing similar increases of expression (data not shown).

found in NF-κB $p50^{-/-}$ and NF-κB $p50^{+/-}$ mice after infection with the natural H5N1 field isolate. We next asked how the lack of hypercytokinemia would influence the pathogenesis in these animals after H5N1 influenza virus infection.

First, the 50% lethal infectious dose was determined (20) for wild-type, $p50^{+/-}$, and $p50^{-/-}$ mice. Interestingly, H5N1 infection of wild-type mice and both NF-κB p50-deficient strains revealed similar 50% lethal dose titers, of 1.7×10^3 PFU for $p50^{+/+}$ mice, 2.1×10^3 PFU for $p50^{+/-}$ mice, and 1.0×10^3

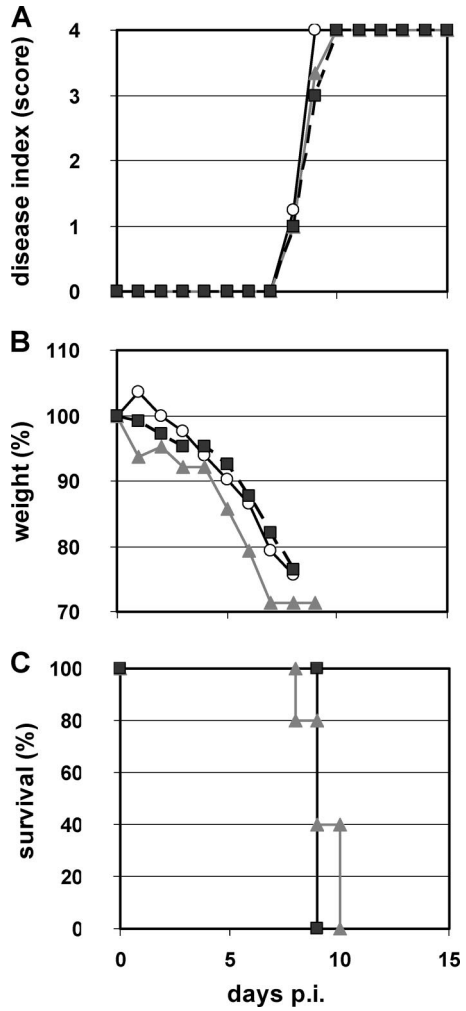

FIG. 2. Disease indexes (A), weights (B), and survival rates (C) after H5N1 infection. Five female NF-κB $p50^{+/+}$ (black squares), $p50^{+/-}$ (gray triangles), or $p50^{-/-}$ (open circles) mice at the age of 6 to 8 weeks, obtained from the animal breeding facilities at the Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Tübingen, Germany, were infected intranasally with 5×10^3 PFU H5N1 (A/mallard/Bavaria/2006) virus. For the estimation of the disease index, animals were scored with different points depending on their health status. Healthy animals scored 0, animals with one symptom (ruffled fur, reduction of normal activity rate, unnatural posture, or fast breathing rate) scored 1, animals with two symptoms scored 2, animals with three or more symptoms scored 3, and dead animals scored 4 for the rest of the 14-day observation period. The graphs represent the mean values for five animals. This experiment was performed twice with comparable results.

PFU for $p50^{-/-}$ mice. For further experiments, all three mouse strains were infected via the intranasal route with 5×10^3 PFU MB1 (H5N1). The mice were weighed and monitored every day. According to German animal protection law, mice were sacrificed when they lost 25 to 30% of their initial body weight. In addition to weight progress and survival rates, the onset and severity of disease were monitored. Surprisingly, there were no differences in the onset of disease between the three mouse strains. All mice developed the first disease symptoms by day 7 postinfection (p.i.). Within a few days, the symptoms became severe and resulted in high disease scores for all three mouse strains (Fig. 2A). Furthermore, all groups of mice showed similar changes in body weight. The animals lost weight immediately after infection. Within 9 days, all mice had lost 30% of their initial body weight (Fig. 2B). The severe symptoms accompanied by weight loss finally resulted in death. Wild-type and $p50^{-/-}$ mice died by day 9 p.i., and $p50^{+/-}$ mice succumbed to the infection within 8 to 10 days after H5N1 inoculation (Fig. 2C).

Next, we questioned whether viral tropism and virus loads were altered after H5N1 infection. Therefore, we performed virus titration of the organs by the Avicel method (16). Mice were infected with 5×10^3 PFU, and the distribution of the virus in the organs was investigated 6 days after infection. Nearly the same virus loads were detected in the lungs, blood, livers, spleens, hearts, and kidneys of infected animals. Interestingly, no virus was detected in the brains (Table 1). This experiment revealed no differences in viral load and viral tropism in H5N1-infected NF-κB $p50^{+/-}$ or NF-κB $p50^{-/-}$ mice compared to wild-type controls. As reported previously, influenza A viruses need activation of the NF-κB signaling pathway, resulting in activation of TRAIL and FasL. Furthermore, caspase-3 activity is required for efficient nuclear export of the viral RNP complex (13, 28, 29). Using real-time reverse transcription-PCR, we demonstrated that TRAIL (Fig. 3A)- and FasL (Fig. 3B)-specific nucleic acids were found in all mouse strains after H5N1 infection, even though the amounts of TRAIL and FasL were slightly reduced in NF-κB $p50^{+/-}$ and NF-κB $p50^{-/-}$ mice. Nevertheless, TRAIL and FasL expression was sufficient to induce caspase-3 activity in the lungs of NF-κB $p50^{+/-}$ and NF-κB $p50^{-/-}$ mice, as indicated by Western blot analysis (Fig. 3C). These results demonstrate that the NF-κB p50 subunit has no influence on TRAIL, FasL, and caspase-3, all of which are required for efficient viral replication in vivo.

Since the transcription factor NF-κB is involved in the regulation of innate and adaptive immunity, we questioned whether a lack of NF-κB would influence the immune cell population after H5N1 infection. Therefore, we investigated the numbers of lymphocytes in the blood of H5N1 influenza

TABLE 1. Distribution of MB1 H5N1 influenza virus after infection of NF-κB p50-deficient mice

Mouse group ($n = 4$)	Virus titer ($\log_{10}$ PFU/ml 10% organ homogenate) at day 6 p.i.[a]						
	Lung	Brain	Kidney	Liver	Spleen	Blood	Heart
$p50^{+/+}$	4.8 ± 0.3	<1.7	3.2 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	4.2 ± 0.2	2.4 ± 0.7
$p50^{+/-}$	5.2 ± 0.3	<1.7	3.1 ± 0.2	3.4 ± 0.1	3.5 ± 0.1	4.0 ± 0.2	2.6 ± 0.1
$p50^{-/-}$	5.2 ± 0.1	<1.7	2.9 ± 0.4	2.8 ± 0.2	3.6 ± 0.1	3.6 ± 0.2	2.1 ± 0.4

[a] The infectious dose was 5×10^3 PFU.

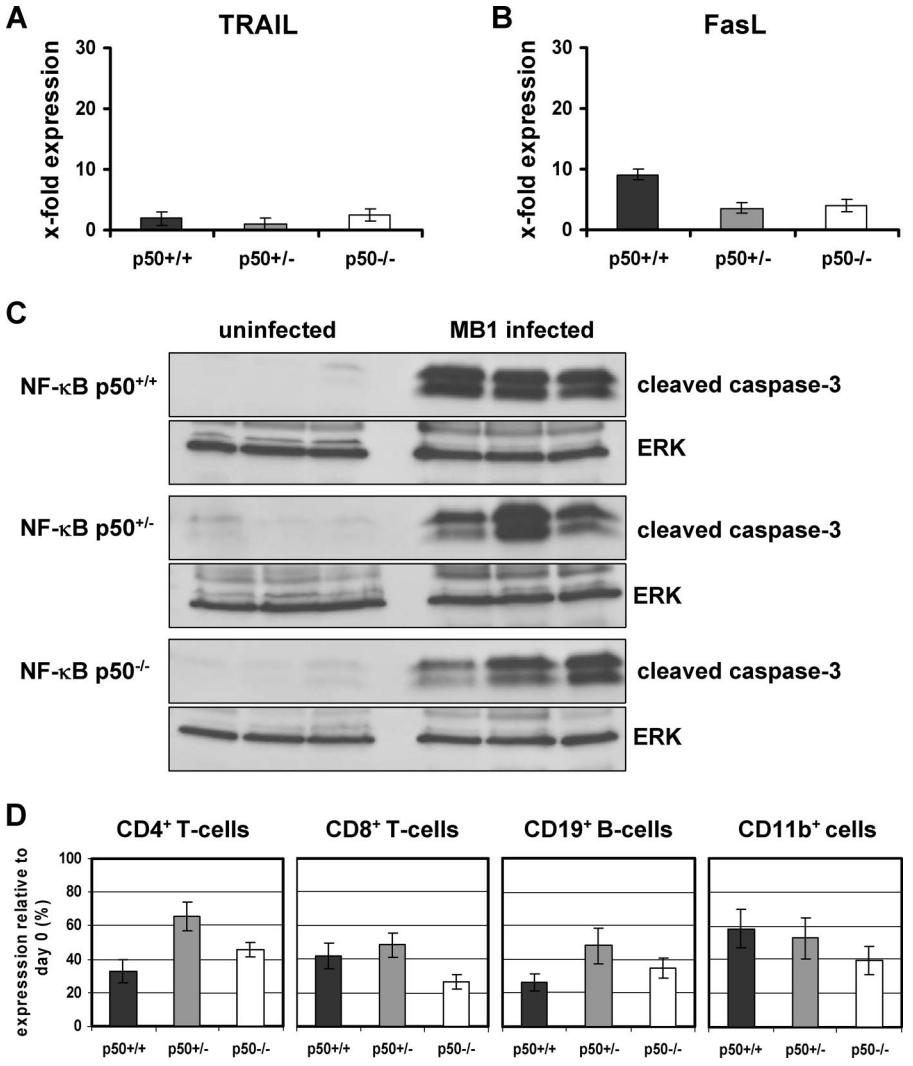

FIG. 3. mRNA levels of TRAIL (A) and FasL (B) in lungs of NF-κB $p50^{+/+}$ (black bars), $p50^{+/-}$ (gray bars) and $p50^{-/-}$ (white bars) mice at 6 days p.i. with 5×10^3 PFU H5N1 virus relative to those in uninfected mice after a reverse transcriptase real-time PCR performed according to the manufacturer's protocol (Qiagen). (C) Western blot analysis for the detection of cleaved caspase-3 (anti-cleaved caspase-3 antibody [Cell Signaling]) in lungs of uninfected (left) and MB1 (H5N1)-infected (right) NF-κB $p50^{+/+}$, $p50^{+/-}$, and $p50^{-/-}$ mice at 6 days p.i. Detection of ERK (anti-ERK; Santa Cruz Biotechnology) was used as a loading control. (D) Lymphopenia after H5N1 infection. Whole blood samples were stained with fluorescent antibodies against CD3, CD4, CD8, CD19, and CD11b according to the manufacturer's protocol (BD Pharmingen). Reductions of $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and $CD11b^+$ cells in the blood of infected NF-κB $p50^{+/+}$ (black bars), $p50^{+/-}$ (gray bars), and $p50^{-/-}$ (white bars) mice at 6 days p.i. with 5×10^3 PFU H5N1 virus are shown relative to the levels in uninfected mice. Numbers of lymphocytes in the blood of uninfected mice were set at 100%. The bars represent the mean values for four animals. There were no significant differences in the reduction of lymphocytes between the NF-κB p50-deficient mouse strains and C57BL/6 controls ($P > 0.1$). This experiment was performed twice with similar results.

virus-infected wild-type and NF-κB p50-deficient mice. Whole blood samples were stained with fluorescent antibodies to detect $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, and $CD11b^+$ monocytes. The number of cells at 6 days p.i. was given as a relative percentage of the number of cells prior to infection. One hallmark of influenza A virus infection is an alteration in the lymphocyte population, also known as lymphopenia (7, 26). Consequently, one might expect a reduction of lymphocytes already in wild-type mice after H5N1 infection. All three mouse strains showed similar reductions of $CD4^+$ T cells after the infection. Even though NF-κB $p50^{+/-}$ (Fig. 3, gray bars) and $p50^{-/-}$ (Fig. 3, white bars) mice had increased numbers of $CD4^+$ T cells compared to wild-type controls (Fig. 3, black bars), Student's *t* test revealed no significant differences in the numbers of $CD4^+$ T cells. Similar reductions of $CD8^+$ T cells, $CD19^+$ B cells, and $CD11b^+$ cells (Fig. 3D) were observed in all three mouse strains 6 days after H5N1 infection. To summarize the data on disease outcome, viral load, and lymphopenia, there was hardly any difference in the pathogenesis between wild-type and NF-κB p50-deficient mice after infection with the H5N1 natural field isolate.

The results revealed no involvement of hypercytokinemia or cytokine storm in H5N1-mediated pathogenesis. From our data, one might argue that inhibition of the local cytokine

response in the lung is not sufficient to reduce the lethality and morbidity after infection with highly pathogenic avian influenza virus. Consequently, our data support the view that early interference with viral replication might be a more promising therapeutic strategy in supporting host survival after infection with a highly pathogenic influenza virus than inhibition of the local cytokine response in the lung by glucocorticoid treatment (22). Moreover, glucocorticoids inhibit the proliferation of antigen-specific plasma B cells (1), which are crucial for controlling the viral spread to organs.

Using a transgenic mouse technology that allows inhibition of the cytokine response and a natural H5N1 field isolate for our investigations, we cannot totally exclude the influence of cytokine storm on H5N1-mediated pathology in humans, in particular because influenza viruses easily mutate. Thus, an antiviral approach that inhibits virus replication and interferes with cytokine expression might be very promising for future therapeutic strategies. Previously, we published a study showing that influenza virus replication is dependent on the activation of the NF-κB signaling pathway (28) and that the NF-κB inhibitor ASA efficiently blocks influenza virus replication both in vitro and in vivo (17). In addition, ASA interacts with a second immunoregulatory pathway. It induces the proteasomal degradation of TNF receptor-associated factor 2 (TRAF2) and TRAF6, leading to diminished cytokine production (14). A recent publication identified the involvement of the Toll-like receptor 4–TRIF–TRAF6 pathway in cytokine production and lung injury after inoculation with inactivated H1N1 or H5N1 viruses, revealing a potential role for oxidative stress and the innate immune response as key regulators of lung injury. Blocking the Toll-like receptor 4–TRIF–TRAF6 pathway resulted in minor lung pathology (10). Thus, antiviral drugs that also target cellular components, in particular the NF-κB signaling pathway, might be candidates for future therapeutic strategies against human infection with highly pathogenic influenza A viruses.

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REFERENCES

1. **Akdis, C. A., T. Blesken, M. Akdis, S. S. Alkan, C. H. Heusser, and K. Blaser.** 1997. Glucocorticoids inhibit human antigen-specific and enhance total IgE and IgG4 production due to differential effects on T and B cells in vitro. Eur. J. Immunol. **27:**2351–2357.
2. **Beigel, J. H., J. Farrar, A. M. Han, F. G. Hayden, R. Hyer, M. D. de Jong, S. Lochindarat, T. K. Nguyen, T. H. Nguyen, T. H. Tran, A. Nicoll, S. Touch, and K. Y. Yuen.** 2005. Avian influenza A (H5N1) infection in humans. N. Engl. J. Med. **353:**1374–1385.
3. **Boeuf, P., I. Vigan-Womas, D. Jublot, S. Loizon, J. C. Barale, B. D. Akanmori, O. Mercereau-Puijalon, and C. Behr.** 2005. CyProQuant-PCR: a real time RT-PCR technique for profiling human cytokines, based on external RNA standards, readily automatable for clinical use. BMC Immunol. **6:**5.
4. **Bonizzi, G., and M. Karin.** 2004. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol. **25:**280–288.
5. **Chan, M. C., C. Y. Cheung, W. H. Chui, S. W. Tsao, J. M. Nicholls, Y. O. Chan, R. W. Chan, H. T. Long, L. L. Poon, Y. Guan, and J. S. Peiris.** 2005. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. Respir. Res. **6:**135.
6. **Cheung, C. Y., L. L. Poon, A. S. Lau, W. Luk, Y. L. Lau, K. F. Shortridge, S. Gordon, Y. Guan, and J. S. Peiris.** 2002. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? Lancet **360:**1831–1837.
7. **Criswell, B. S., R. B. Couch, S. B. Greenberg, and S. L. Kimzey.** 1979. The lymphocyte response to influenza in humans. Am. Rev. Respir. Dis. **120:**700–704.
8. **de Jong, M. D., C. P. Simmons, T. T. Thanh, V. M. Hien, G. J. Smith, T. N. Chau, D. M. Hoang, N. V. Chau, T. H. Khanh, V. C. Dong, P. T. Qui, B. V. Cam, D. Q. Ha, Y. Guan, J. S. Peiris, N. T. Chinh, T. T. Hien, and J. Farrar.** 2006. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat. Med. **12:**1203–1207.
9. **Hayden, F. G., R. Fritz, M. C. Lobo, W. Alvord, W. Strober, and S. E. Straus.** 1998. Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. J. Clin. Investig. **101:**643–649.
10. **Imai, Y., K. Kuba, G. G. Neely, R. Yaghubian-Malhami, T. Perkmann, G. van Loo, M. Ermolaeva, R. Veldhuizen, Y. H. Leung, H. Wang, H. Liu, Y. Sun, M. Pasparakis, M. Kopf, C. Mech, S. Bavari, J. S. Peiris, A. S. Slutsky, S. Akira, M. Hultqvist, R. Holmdahl, J. Nicholls, C. Jiang, C. J. Binder, and J. M. Penninger.** 2008. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell **133:**235–249.
11. **Kobasa, D., S. M. Jones, K. Shinya, J. C. Kash, J. Copps, H. Ebihara, Y. Hatta, J. H. Kim, P. Halfmann, M. Hatta, F. Feldmann, J. B. Alimonti, L. Fernando, Y. Li, M. G. Katze, H. Feldmann, and Y. Kawaoka.** 2007. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature **445:**319–323.
12. **Lee, N., C. K. Wong, P. K. Chan, S. W. Lun, G. Lui, B. Wong, D. S. Hui, C. W. Lam, C. S. Cockram, K. W. Choi, A. C. Yeung, J. W. Tang, and J. J. Sung.** 2007. Hypercytokinemia and hyperactivation of phospho-p38 mitogen-activated protein kinase in severe human influenza A virus infection. Clin. Infect. Dis. **45:**723–731.
13. **Ludwig, S., S. Pleschka, O. Planz, and T. Wolff.** 2006. Ringing the alarm bells: signalling and apoptosis in influenza virus infected cells. Cell. Microbiol. **8:**375–386.
14. **Machado, F. S., L. Esper, A. Dias, R. Madan, Y. Gu, D. Hildeman, C. N. Serhan, C. L. Karp, and J. Aliberti.** 2008. Native and aspirin-triggered lipoxins control innate immunity by inducing proteasomal degradation of TRAF6. J. Exp. Med. **205:**1077–1086.
15. **Maines, T. R., X. H. Lu, S. M. Erb, L. Edwards, J. Guarner, P. W. Greer, D. C. Nguyen, K. J. Szretter, L. M. Chen, P. Thawatsupha, M. Chittaganpitch, S. Waicharoen, D. T. Nguyen, T. Nguyen, H. H. Nguyen, J. H. Kim, L. T. Hoang, C. Kang, L. S. Phuong, W. Lim, S. Zaki, R. O. Donis, N. J. Cox, J. M. Katz, and T. M. Tumpey.** 2005. Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. J. Virol. **79:**11788–11800.
16. **Matrosovich, M., T. Matrosovich, W. Garten, and H. D. Klenk.** 2006. New low-viscosity overlay medium for viral plaque assays. Virol. J. **3:**63.
17. **Mazur, I., W. J. Wurzer, C. Ehrhardt, S. Pleschka, P. Puthavathana, T. Silberzahn, T. Wolff, O. Planz, and S. Ludwig.** 2007. Acetylsalicylic acid (ASA) blocks influenza virus propagation via its NF-kappaB-inhibiting activity. Cell. Microbiol. **9:**1683–1694.
18. **Pahl, H. L.** 1999. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene **18:**6853–6866.
19. **Peiris, J. S., W. C. Yu, C. W. Leung, C. Y. Cheung, W. F. Ng, J. M. Nicholls, T. K. Ng, K. H. Chan, S. T. Lai, W. L. Lim, K. Y. Yuen, and Y. Guan.** 2004. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet **363:**617–619.
20. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Epidemiol. **27:**493–497.
21. **Rimmelzwaan, G. F., T. Kuiken, G. van Amerongen, T. M. Bestebroer, R. A. Fouchier, and A. D. Osterhaus.** 2001. Pathogenesis of influenza A (H5N1) virus infection in a primate model. J. Virol. **75:**6687–6691.
22. **Salomon, R., E. Hoffmann, and R. G. Webster.** 2007. Inhibition of the cytokine response does not protect against lethal H5N1 influenza infection. Proc. Natl. Acad. Sci. USA **104:**12479–12481.
23. **Sandrock, C., and T. Kelly.** 2007. Clinical review: update of avian influenza A infections in humans. Crit. Care **11:**209.
24. **Sladkova, T., and F. Kostolansky.** 2006. The role of cytokines in the immune response to influenza A virus infection. Acta Virol. **50:**151–162.
25. **Szretter, K. J., S. Gangappa, X. Lu, C. Smith, W. J. Shieh, S. R. Zaki, S. Sambhara, T. M. Tumpey, and J. M. Katz.** 2007. Role of host cytokine

responses in the pathogenesis of avian H5N1 influenza viruses in mice. J. Virol. **81:**2736–2744.

26. **Tran, T. H., T. L. Nguyen, T. D. Nguyen, T. S. Luong, P. M. Pham, V. C. Nguyen, T. S. Pham, C. D. Vo, T. Q. Le, T. T. Ngo, B. K. Dao, P. P. Le, T. T. Nguyen, T. L. Hoang, V. T. Cao, T. G. Le, D. T. Nguyen, H. N. Le, K. T. Nguyen, H. S. Le, V. T. Le, D. Christiane, T. T. Tran, D. J. Menno, C. Schultsz, P. Cheng, W. Lim, P. Horby, and J. Farrar.** 2004. Avian influenza A (H5N1) in 10 patients in Vietnam. N. Engl. J. Med. **350:** 1179–1188.
27. **Wareing, M. D., A. B. Lyon, B. Lu, C. Gerard, and S. R. Sarawar.** 2004. Chemokine expression during the development and resolution of a pulmonary leukocyte response to influenza A virus infection in mice. J. Leukoc. Biol. **76:**886–895.
28. **Wurzer, W. J., C. Ehrhardt, S. Pleschka, F. Berberich-Siebelt, T. Wolff, H. Walczak, O. Planz, and S. Ludwig.** 2004. NF-kappaB-dependent induction of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Fas/FasL is crucial for efficient influenza virus propagation. J. Biol. Chem. **279:**30931–30937.
29. **Wurzer, W. J., O. Planz, C. Ehrhardt, M. Giner, T. Silberzahn, S. Pleschka, and S. Ludwig.** 2003. Caspase 3 activation is essential for efficient influenza virus propagation. EMBO J. **22:**2717–2728.
30. **Zitzow, L. A., T. Rowe, T. Morken, W. J. Shieh, S. Zaki, and J. M. Katz.** 2002. Pathogenesis of avian influenza A (H5N1) viruses in ferrets. J. Virol. **76:** 4420–4429.